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**Olive oil and nuts among two ethnic
groups: an evaluation of their
acceptability, a survey and systematic
review of previous interventional
evidence of their effects on
cardiovascular health, and results from
an RCT intervention study**

F LIANG

PhD

2021

Olive oil and nuts among two ethnic groups: an evaluation of their acceptability, a survey and systematic review of previous interventional evidence of their effects on cardiovascular health, and results from an RCT intervention study

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A thesis submitted in partial fulfilment of the requirements of the University of Northumbria at Newcastle for the degree of Doctor of Philosophy

Research undertaken in the Faculty of Health and Life Sciences

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ABSTRACT

Among the physiological and metabolic changes occurring with ageing, the ageing of heart function is a key determinant of health. The death number from CVDs is expected to reach over 23.6 million by 2030. An estimated 17.9 million people died from CVDs in 2019 in the UK, representing 32% of all global deaths. Evidence suggested that the Mediterranean diet supplemented with extra virgin olive oil (EVOO) (25-50 ml/day) is highly reported as associated with a reduction of CV risk factors. However, the acceptability of the Mediterranean diet and the feasibility of this dietary pattern which includes consumption of olive oil remains unknown among Caucasians and East Asians in Northeast England. An Online Survey with two ethnicities in equal number and similar mean age and BMI that were undertaken for this PhD programme indicating that the acceptability and frequency of olive oil intake among East Asians is higher with a great MD score ($8.02 \pm SD1.8$) ($p < 0.001$) while Caucasians who consume olive oil were scored higher for MD score ($6.51 \pm SD2.2$) ($p < 0.001$), scored higher for MD acceptability ($10.21 \pm SD2.3$) ($p = 0.017$) and reported lower perceived barriers to healthy eating (PBHE) ($1.81 \pm SD4.0$) ($p = 0.03$) than non-consumers. Olive oil intake is likely to be positively associated with older age, higher MD score, higher MD acceptability and lower PBHE in both ethnicities. Evidence examining the effectiveness of nuts and olive oil, on both traditional and novel CV risk factors, in a comprehensive study in adults with different ethnic background is lacking. Our systematic reviews and meta-analysis of previous relevant literature on nuts that were undertaken for this PhD programme showed that nuts improve TC (MD: -7.54; 95% CI: -10.2 to -4.89; $p < 0.00001$; $I^2 = 59\%$, $n = 66$), HDL (MD: 0.89; 95% CI: 0.04 to 1.75; $P = 0.04$; $I^2 = 53\%$; $n = 67$), LDL (MD: -7.21; 95% CI: -9.38 to -5.04; $P < 0.00001$; $I^2 = 68\%$; $n = 68$), TG (MD: -8.83; 95% CI: -13.12 to -4.53; $P < 0.0001$; $I^2 = 64\%$; $n = 65$) and FMD (MD: 0.74; 95% CI: 0.09 to 1.39; $P = 0.03$; $I^2 = 5\%$, $n = 10$). The non-Asian group potentially tends to benefit more CV biomarkers with moderate nut consumption than Asian group. Olive oil systematic review reported that olive oil improves biomarker - PAI-1 (MD: -1.02ng/ml, 95% CI: -1.92 to -0.12; $p = 0.03$, $I^2 = 0\%$). Nevertheless, studies on olive oil on different ethnicities were lacking. A 6-week, cross-over, randomised controlled dietary interventional study with 2 weeks interventional duration was undertaken to test the effects of EVOO on cardiovascular health. Overall, this study provided evidence on the benefits of over a 2-week period produced a positive effect on 24-hour SBP including daytime SBP, night-time DBP and MAP and TC, LDL for all participants. For East Asians, olive oil exerts a beneficial effect on 24-hour SBP and daytime SBP, MAP while night-time DBP was improved among Caucasians following EVOO. EVOO intake also has a positive effect on blood lipids - TC and circulating biomarkers - sE-selectin in East Asians while LDL and non-HDL are improved among Caucasians after EVOO intake. The findings reported in the present thesis could be valuable to health professionals to develop more effective interventions and could also help the public to make better informed food choices relating to cardiovascular health.

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DECLARATION

I declare that the work contained in this thesis has not been submitted for any other award and that it is all my own work. I also confirm that this work fully acknowledges opinions, ideas and contributions from the work of others.

Any ethical clearance for the research presented in this thesis has been approved. Olive oil clinical trial approval has been sought and granted by the Northumbria University Ethics Committee (**Project ref: 10527**) on 6th November 2018. Survey approval has been sought and granted by the Northumbria University Ethics Committee (**Project ref: 892**) on 28th May 2019.

I declare that the Word Count of this Thesis is 59,521 words

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ABBREVIATIONS

ABP	Ambulatory blood pressure
AF	Atrial fibrillation
AHA	American Heart Association
ALA	α -linolenic acid
Apo A1	Apolipoprotein A1
Apo B	Apolipoprotein B
BMI	Body mass index
BIA	Bioelectrical impedance analysis
BP	Blood pressure
CAD	Coronary artery disease
CCTR	Cochrane Central Register of Controlled Trials
CDSR	Cochrane Database of Systematic Review
CHD	Coronary heart disease
CONSORT	Consolidated Standards of Reporting Trials
CI	Confidence interval
CRP	C-reactive protein
CVDs	Cardiovascular diseases
°C	Degrees centigrade
DBP	Diastolic blood pressure
DHA	Docosahexaenoic acid
ECs	Endothelial cells
EI	Energy intake
sE-selectin	Soluble E-selectin
ET-1	Endothelial-1
EF	Endothelial function
eNOS	Endothelial nitric oxide synthase
EPA	Eicosapentaenoic acid
EVOO	Extra virgin olive oil
ESRC	Economic Social Research Council
FFA	Free fatty acid
FMD	Flow-mediated dilation
HCD	High cholesterol diet
HDL	High density lipoprotein

HR	Heart rate
HT	Hydroxytyrosol
IHD	Ischaemic heart disease
IL-6	Interleukin-6
LDL	Low-density lipoprotein
LFHC	Low-fat-high-carbohydrate
MD	Mediterranean diet
MDS	Mediterranean diet score
MET	Metabolic equivalents
mg	Milligram
min	minute (s)
mL	Milliliter (s)
mmHg	Millimeter of mercury
mmol	Millimole
MMPs	Mitochondrial membrane potential
MI	Myocardial infarction
MUFA	Monounsaturated fatty acids
NO	Nitric oxide
Ox-LDL	Oxidized low-density lipoprotein
PAD	Peripheral arterial disease
PAI-1	Plasminogen activator inhibitor
PBHE	Perceived barriers to healthy eating
PRISMA	Preferred Reporting Items for Systematic Reviews and Meta-Analyses
PREDIMED	PREvención con Dieta MEDiterránea
PROSPERO	International prospective register of systematic reviews
sP-selectin	Soluble P-selectin
PUFA	Polyunsaturated fatty acids
PWV	Pulse wave velocity
RCTs	Randomised controlled trials
ROO	Refined olive oil
RRs	Risk ratios
SBP	Systolic blood pressure
SD	Standard deviation
SEM	Standard error of mean
SFA	Saturated fatty acid

sICAM-1	Soluble intercellular adhesion molecules 1
SMD	Standardized mean differences
sVCAM-1	Soluble vascular cell adhesion molecules 1
TC	Total cholesterol
tPA	Tissue plasminogen activator
TG	Triglyceride
TNF-α	Tumour necrosis factor alpha
VWf	Von Willebrand factor
WC	Waist circumference
WHtR	Waist-to-height ratio
μg	Microgram (s)
μL	Microliter (s)

Conferences

Original Communications in Academic and Scientific

1. The Nutrition Summer Society Student Conference, 10 - 12 July 2018: Getting energy balance right. The Riverside Innovation Centre, University of Chester, UK; Proceedings of The Nutrition Society 77(OCE4); F Liang, J Young, J Lara. "Efficacy of nutritional interventions supplementing nuts on cardiovascular risk factors among adults individuals: A systematic review and meta-analysis of intervention studies" Volume 77 Issue OCE4

Awards

From The Trustees of the Rank Prize Funds

Fan Liang was nominated by Department of Applied Sciences of Northumbria university to the Trustees of the Rank Prize Funds during the COVID-19 pandemic in 2020, and Fan was favorably awarded 5000£ for supporting her PhD dietary research topic.

As a recent Rank Prize grant winner, Fan Liang was honored to be invited to join to celebrate 50 years of Rank Prize at the Royal College of Physicians during 2022. It is a great opportunity for Fan a dietary researcher to improve skills and make further career in this event.

CHAPTER 1

Introduction

1 Cardiovascular health and CVDs in Britain and East Asia

1.1 cardiovascular disease

Cardiovascular disease (CVDs) encompasses all the conditions concerning the heart and circulatory systems. CVDs refers to the degeneration and interruption of arteries and inadequate supply of oxygen to the muscle of the heart - coronary heart disease (CHD), the brain including cerebrovascular and carotid artery disease and the extremities, particularly the lower limbs (peripheral vascular disease). CVD involves the processes of atherosclerosis (lesions in the arterial wall) and thrombosis (blood clotting) as well as changes to the function of the arterial lining (American Heart Association 2019). Atherosclerosis is a complex disease of the arteries, as fatty materials called atheroma builds up in the lining of artery walls and narrows arteries. Over time it grows bigger until the passageways through the arteries become roughened and clogged with fatty deposits that they cannot left enough blood flow freely (Lusis, 2000).

1.2 Epidemiologic characterization for cardiovascular health

In the early 20th century, CVDs were responsible for nearly 10% of all deaths globally. At the beginning of the 21st century, CVDs account for nearly one half of all deaths in the developed world and 25% of all deaths in developing countries (Sokoła-Wysoczańska, Wysoczański et al. 2018). Evidence (Townsend, Wilson et al. 2016) suggested that CVD remain the leading cause and accounts for 17.3 million deaths per year. The mortality of CVD in Europe accounted for 45% of all deaths - 49% among women and 40% among men. Over 4 million people die from CVDs across Europe annually, with 1.4 million of these deaths before the age of 75. The death number from CVDs is expected to reach over 23.6 million by 2030 (World Health Organization 2020).

Study reported that the majority of patients with coronavirus (COVID-19) have underlying CVD as 7% of patients experience myocardial injury from the infection (22% of critically ill patients) (Clerkin, Fried et al. 2020) and acute cardiac injury was reported to become the most reported cardiovascular abnormality during the pandemic of coronavirus (COVID-19), with average incidence of 8-12% (Bansal 2020). Another current research news found that

UK citizen suffering with health conditions such as heart disease, stroke and other their risk factors such as diabetes, obesity, hypertension is at increased risk of complications from coronavirus and the increased risk of COVID-19 complications in cardiovascular disease to an abundance of a receptor that serves as a gateway for the virus to jump into the lungs and heart as evidence indicated that 45% of the Covid-19 deaths in English citizens who also had a history of cardiovascular diseases (British Heart Foundation 2021). Review study has been observed that mortality rates in patients of coronavirus disease 2019 (COVID-19) have been examined approximately 10.5% cases in patients with cardiovascular disease and a mortality rate of 52% was recorded in patients with heart failure, while 12% recovered ultimately (Dan et al., 2020). In China, a meta-analysis comprising Chinese patients showed that pre-existing CVD was associated with a significantly increased risk of a severe form of COVID-19 (OR = 3.14; 95% CI: 2.32-4.24; $I^2 = 0\%$; $Q = 8.68$, $P = 0.73$) and overall risk of COVID-19 all-cause mortality (OR = 11.08; 95% CI: 2.59-47.32; $I^2 = 55\%$; $P = 0.11$) (Aggarwal, Cheruiyot et al. 2020). The current urgent exposure to CVD risk factors is highly impacted by the environment in which an individual lifestyle including dietary consumption, which means that the majority of CVDs result from risk factors that may be controlled and treated, which include hypertension, cholesterol, diabetes, tobacco use, lack of physical activity, and overweight/obesity (Sokoła-Wysoczańska, Wysoczański et al. 2018).

1.2.1 CVD in the UK – emphasis in the North East of England

Around 7.6 million people - 4 million males and 3.6 million females live with CVD in the UK and around 650,000 people of those have heart failure while 1.9 million people are living with coronary heart disease (CHD), which is responsible for around 63,000 deaths in the UK each year. Around 15 million adults (28% of adults) experience high blood pressure in the UK (British Heart Foundation 2021).

Between 2015 and 2017, almost 30% of all North East residents dying from CVD were aged under 75 years (Public Health England 2019) and the rate of premature CVD mortality in NE was the second highest of all the English regions and significantly higher than the national rate (Valerie et al, 2020). The gap in the under 75 age-standardised CVD mortality rate between the North East and England has narrowed over the past 15 years however the rates in the North East is still significantly (14%) greater than the England rate (Public Health England 2019). By 2030, the population in England aged 65-84y will potentially rise by 29% and those over 85 by 61%. Treatment and care for people with long term conditions

is estimated at £7.4 billion per year, and annual costs to the wider economy being an estimated £15.8 billion in the UK (Public Health England 2019).

In addition, evidence (Public Health England 2019) showed that an estimated 81% of Disability-adjusted Life Years (DALYs) due to CVD in the North East England can be attributed to cardiovascular risk factors. 71% of DALYs are the result of cardiovascular risk factors such as hypertension or high body mass index (BMI). 55% of DALYs are attributable to behavioural risks, such as poor diet or smoking habits (Public Health England 2019). Evidence estimated that 60% of North East English citizens have a raised cholesterol level (Waterall 2015). Moreover, evidence also indicated that 1 in 250 and 1 in 500 persons experience familial hypercholesterolaemia, which is a genetic reason of raised cholesterol levels (Public Health England 2019).

1.2.2 CVDs in East Asia – emphasis in China

East Asians have an increased risk of death from overall CVDs (Chen, Copeland et al. 2013). The incidence of CVDs among Chinese adults are lower than that found in most economically developed countries, however it had risen in a strikingly fast rate over the past several decades (Zhao, Chong et al. 2001). In China, the upwards tendency of the proportion of patients with CVD were closely associated with the increased number of cardiovascular risk factors and population ageing (Li and Ge 2014). The prevalence of cardiovascular risk factors such as dyslipidemia was 62.1% overall, with 33.5% high total cholesterol (TC), 43.9% triglyceride (TG), 0.6% low-density lipoprotein cholesterol (LDL-C) and 8.8% for low high-density lipoprotein cholesterol (HDL-C). In those with dyslipidemia in China, the proportion of Chinese subjects who were aware, treated, and controlled was low, which is 14.4, 33.9, and 19.9%, respectively (Zhang, Xing et al. 2017). A cross-sectional study (Zhu, Xi et al. 2020) with 56,716 Chinese residents aged 40 years and above from six cities in Northern China from September 2015 to June 2017 reported that 22.7% of participants had a high 10-year risk of CVD (Zhu, Xi et al. 2020). Individuals with high socioeconomic status should be encouraged to change their unhealthy lifestyle habits and greater medical resources should be invested for individuals residing in rural areas (Zhu, Xi et al. 2020).

1.3 Definitions of 'ethnic groups'

The UK's Economic Social Research Council (ESRC) refers to ethnic groups as 'people of the same race or nationality with a long shared history and a distinct culture' and defined ethnicity as the 'intangible quality, or sense of being, derived from that shared racial or cultural affiliation' (Leung and Stanner 2011).

1.3.1 Chinese immigration and the prevalence of CVD among Chinese immigration in the UK

The UK has a rich mix of cultures and culturally diverse communities and Chinese is one of the biggest minority ethnic groups (South Asians, Black African-Caribbean and the Chinese) living in the UK (Leung and Stanner 2011). 33% of ethnic Chinese people (120,250) in the United Kingdom reside in London, comprising 1.5% of the city's population. Manchester, second-most populous urban area, is home to the largest Chinese population, with 3.4% of all Chinese people living there. This is followed by Birmingham (3.2%) and Barnet, Tower Hamlets and Southwark (all at 2.1%). British Chinese communities, which are descended from overseas Chinese when they immigrated to the UK, are found in many major UK cities including London, Manchester, Birmingham, Belfast, Newcastle, Glasgow, Edinburgh, Cardiff, Liverpool, Sheffield, Nottingham, and Aberdeen. Among these immigrants, 23.7% of people from the Chinese ethnic group were born in the UK and over half (55.3%) were born in Eastern Asia. 34.5% of adults from the Chinese ethnic group were overweight or obese, which is the lowest percentage out of all ethnic groups (Gov.UK 2020).

It is believed that minority ethnic groups are more likely to experience poorer health outcomes, such as higher rates of CVD, type 2 diabetes and obesity, compared with the mainstream population since migration and mobility to foreign latitudes is believed to be responsible for health differentials between origin and destination and people who migrate are more likely to experience diseases due to exposure to new lifestyles and environment (Cappuccio 2004). The differences in health outcomes also may reflect the interaction between different diet patterns and other health behaviours and genetic predisposition which vary across different ethnic groups (Leung and Stanner 2011).

The health of Chinese immigrants in the UK is controversial. Studies (Gong and Zhao 2015, Jin, Ding et al. 2015) reported that Chinese immigrants, having both higher prevalence and higher mortality rates from CHD compared with mainland Chinese citizens. Chinese immigrants also appear to have a higher prevalence of cardiac risk factors such as hypertension, higher cholesterol, poorer dietary patterns, and higher prevalence of obesity

after migrating to Western countries, and the risk increases with longer length of residence. In the meanwhile, CVD in Chinese immigrants also have had consistently higher stroke mortality (Dassanayake, Gurrin et al. 2011). However, while Chinese people had a lower incidence of CHD compared with white Caucasians (odds ratio 0.29; 95% CI: 0.24–0.34) but higher short-term mortality after first hospitalization for acute myocardial infarction compared with whites (odds ratio 1.34; 95% CI, 1.04–1.73) (Jin, Ding et al. 2015). Another study from Newcastle upon Tyne reported that in a cross-sectional study of 380 Chinese and 625 European adults aged around 25 to 64 years old, there was significantly lower ($P < 0.001$) CHD prevalence among Chinese (4.9%) compared to European origin men (16.6%) (Frouhi and Sattar 2006). However, there is still a lack of enough research focusing on CVD status among Chinese immigrant groups.

1.4 Non-modifiable factor - Genetic factors affect cardiovascular health

Gene-environment interaction impacts on cardiovascular health and genetic factors are irreversible (Kelishadi and Poursafa 2014) and evidence (Kelishadi and Poursafa 2014) suggested that the ethnic disparities in cardiovascular risk varies is due to genetic differences. A research of susceptibility loci for coronary artery disease suggested that 4 loci associating with coronary artery disease were common amongst the Han Chinese population and European populations, 4 were unique to the Han Chinese (Lu, Wang et al. 2012). Different ethnicity background determines cultural food preferences, therefore genetic factors contribute to cultural cuisine preferences, plus modulate the association between dietary components and adverse cardiovascular health outcomes (Leu, Chung et al. 2019).

1.4.1 The difference of body composition between East Asians and Caucasians affecting cardiovascular health

Genetic differences are at the root of different body fat patterns in different ethnic groups-Asians and Caucasians (Harvard T.H. Chan 2021). Different body composition from Caucasians and East Asians determines different effects of dietary interventions (Goss, Goree et al. 2013). Asians were reported to have greater predisposition towards adiposity at higher BMI compared to Caucasians (Haldar, Siok Ching et al. 2015). For the same BMI, Asians tend to have a higher body fat percentage (3% to 5% higher total body fat), a prominent abdominal obesity, a higher intramyocellular lipid and/or a higher liver fat content compared to Caucasians. Similarly, Asians are more likely to have a much greater

predisposition to risks of hypertension and cardiovascular diseases than their white European counterparts (Pan, Flegal et al. 2004, Wen, David Cheng et al. 2009, Haldar, Siok Ching et al. 2015, Harvard T.H. Chan 2020).

In China, mainland Chinese males had a greater degree of central fat deposition pattern than that Caucasians men (Wang, Li et al. 2011). Chinese males had significantly higher percentage of body fat both with respect to entire body (Chinese: 23.7%±0.2% vs. Caucasians:22.4%±0.2%) and the trunk area (Chinese: 25.0%±0.3% vs. Caucasians: 23.2%±0.3%) compared to their Caucasian counterparts. At all same BMIs, Chinese men were reported as having significantly higher fasting glucose levels (Chinese: 5.7±1.0 mmol/L vs. Caucasians: 5.2±1.0 mmol/L) but lower HDL-C levels (Chinese: 0.8±1.0 mmol/L vs. Caucasians: 1.0±1.0 mmol/L) than Caucasian men (Wang, Li et al. 2011).

Evidence (Harvard T.H. Chan 2021) suggested that for every 11 pounds Asians gained in their adulthood, Asians had an 84 percent increase their risk factors of cardiovascular diseases such as type 2 diabetes. Caucasians who gained weight also experienced higher diabetes risks, however, to a much lesser degree than Asians (Harvard T.H. Chan 2021).

The phenomenon that East Asians gain body weight in Western countries is probably because that even though immigrants might arrive in new countries with a health advantage including generally a healthier body weight. Nevertheless, it was reported that genetic and epi-genetic factors, alongside with body size preference, socio-economic factors and stress exposure, may play an crucial part in growing unhealthy weight gain among migrant populations (Murphy, Robertson et al. 2017). This tendency of unhealthy weight gain is likely to lead to overweight during first few years of migration, and such weight gain tends to lead to increased obesity risk in migrant populations within 10 to 15 years after migration (Murphy, Robertson et al. 2017).

1.4.2. Gender for cardiovascular health

Gender differences of CVDs are mainly caused by innate genes and environmental influences (Gao, Chen et al. 2019). Males are not only reported to have a higher incidence of CVD than females in general (Gao, Chen et al. 2019), but also to have an earlier age development of CVD incidence and a higher propensity of developing coronary heart disease than female (Bots, Peters et al. 2017). Evidence showed that more men have higher rate of living with and dying of coronary heart disease (CHD) than women and men also experienced more hospital discharges for CVD as well as CHD (Mosca, Barrett-Connor et

al. 2011). The prevalence of CHD is higher in men within each age stratum until after 75 years of age (Mosca, Barrett-Connor et al. 2011). In addition, prospective population based study (Millett, Peters et al. 2018) found that almost 472,000 male citizens in the UK aged 40 to 69 had a far higher risk of heart attack than women over 7 years of follow-up and while the incidence of myocardial infarction (MI) was higher in men than in women, some risk factors were more strongly associated with MI in women compared with men.

Lifestyle risk factors also vary by gender (Mosca, Barrett-Connor et al. 2011) as cigarette smoking remains more common among men than women (23.1% versus 18.1%) (Mosca, Barrett-Connor et al. 2011). However, female smokers had about 3.4 times the risk of having a heart attack as women who had never smoked, while male smokers had 2.2 times the risk of men who had never smoked (Millett, Peters et al. 2018).

Nevertheless, study (Eric and I-Min 2010) reported that although the magnitude of association with risks of CVDs appeared stronger in women than in men, comparisons across gender are limited by the fact that the various studies applied varied questionnaires for assessing physical activity combined with diverse categorizations (e.g., by amount of energy expended, intensity of activities, duration, or frequency) of physical activity for analyses.

1.4.3 Ageing from chronological age to vascular age

Human lifespan is restricted, everyone accepts this “biologically” obvious. “Nothing lives forever”, however, in this statement people think of ageing process, which are subject to natural wear and tear during human life. As ageing is a global phenomenon and developing interventions to promote health in early and later life are a priority of health research (Levine et al., 2018), however, evidence (WHO 2018) suggested that the prevalence of healthy ageing was relatively low. By 2030, approximately 20% of the global population, who will be aged 65 or older and in this age group, cardiovascular diseases will result in 40% of all deaths. Also, the cost to treat cardiovascular disease was expected to be triple in that time (North and Sinclair 2012). People become dissimilar from their contemporaries of the same chronological age as they become older mostly due to long-term unhealthy lifestyle factors

The risk for CVD starts around 40-45 years old. People during such age are more likely to experience vascular risk factors such as dyslipidaemia, hypertension or endothelial dysfunction due to unhealthy lifestyle including unhealthy dietary patterns (O'Doherty, Cairns et al. 2016). Statistics showed that heart failure is largely a disease of the elderly

with 50% of all heart failure diagnoses and 90% of all heart failure deaths occurring in the segment of the population over age 70 (Strait and Lakatta 2012).

Aging, as a major risk factor for CVDs, not only increases the prevalence of CVDs but is also associated with impaired responses to CVDs because ageing results in structural changes and functional decline of the cardiovascular system (Fajemiroye, da Cunha et al. 2018). Human biological ageing is inevitable (Vijg and Le Bourg 2017) and chronological age is the most significant non-modifiable risk factor for developing CVDs (Hollis, Newson et al. 2016). Healthy ageing refers to survival to a specific age, being free of chronic diseases, autonomy in activities of daily living, wellbeing, good quality of life, high social participation, only mild cognitive or functional impairment, and little or no disability (Fuchs, Scheidt-Nave et al. 2013). Individuals do not age at the same pace leading to the concept of biological aging, also called functional or physiological ageing, which relates to declines in function (Vijg and Le Bourg 2017). Wide variation exists in ageing among people as characterised by the degree to which body functions decline, including declines in cognition, physical capability, physiological and metabolic functions, and psychosocial wellbeing (Murman 2015). Among the physiological and metabolic changes occurring with biological ageing, the ageing of heart function is the key determinant of health (McPhee, French et al. 2016).

Cardiovascular ageing is accompanied by changes in vascular structure as well as function, especially in the large arteries along with age-related impairment of vascular function is the result of phenotypic alterations of different cell types, such as endothelial cells, smooth muscle cells and pericytes (Donato Anthony, Machin Daniel et al. 2018). Poor vascular ageing is considered to be the most important risk factor affecting cardiovascular homeostasis (Costantino, Paneni et al. 2016).

1.4.4 Body composition factors

Measures such as body mass index (BMI), grip strength and body composition including body mass index, body fat/lean percentage are good indicators of cardiovascular disease mortality risk (Sözmen, Belgin et al. 2016).

Evidence strongly suggested that the likelihood of a greater number of CV risk factors is greater among those who are overweight (body fatness \geq 20%), high BMI, than lean participants (body fat <20%) (Amirabdollahian and Haghghatdoost 2018). The likelihood of multiple CV risk factors is greater among those with high body fatness in apparently healthy young men (Liberato, Maple-Brown et al. 2013). Furthermore, body mass index (BMI), waist

circumference (WC) and waist-to-height ratio (WHtR) are strongly shown to be associated with serum lipid, and TC, TG, LDL-C and HDL-C. Also, BMI, WC and WHtR were closely related to the incidence of dyslipidemia in females, including high TG, high LDL-C and low HDL-C (Zhang, Gu et al. 2019). Hyper LDL cholesterolemia is significantly associated with percent body fat, but not with body mass index or waist circumference, in men (Oda 2017).

Evidence (Beyer, Sanghvi et al. 2018) indicated that handgrip strength, an inexpensive, reproducible and easy to implement measure, is associated with risk for cardiovascular incidents and mortality. Stronger hand grip strength may be associated with cardiac functions and structures that help reduce the risk of cardiovascular incidents (Beyer, Sanghvi et al. 2018). Their study of including over 4,600 people (Beyer, Sanghvi et al. 2018) reported that better handgrip strength is associated with having a healthier heart structure and function as participants with stronger hand grips were often pumping more blood per heartbeat despite having a lower heart mass.

1.5 Modifiable factor - Behavioral risk factors for cardiovascular health

1.5.1 Physical activities

Physical activity is defined as any bodily movement produced by skeletal muscles that requires energy expenditure (World Health Organization 2018). Physical activity refers to movement including during leisure time, for transport to get to and from places, or as part of a individual's work. A physically inactive lifestyle contributes to traditional cardiovascular risk factors, such as high BP, higher triglycerides, lower HDL-C and obesity (Lavie Carl, Ozemek et al. 2019). Previous randomized controlled trials suggested the benefits of aerobic exercise on blood pressure (Seamus and Whelton 2002) as well as cholesterol levels (Durstine and Grandjean 2001). The inverse association between physical activity and risk of developing CVDs is shown in both genders (Eric and I-Min 2010). Physical activity has also been shown to decrease levels of novel cardiovascular risk factors and improve endothelial function (EF), such as IL-6, sICAM-1 and sVCAM-1 (Palmefors, DuttaRoy et al. 2014). Physical activity is associated with a 35% reduction in CVDs mortality and 33% reduction in all-cause mortality in comparison with sedentary lifestyle (Nocon, Hiemann et al. 2008). Study (Lear, Hu et al. 2017) reported that compared with low physical activity (<150 minutes per week of moderate intensity physical activity), moderate (150-750 minutes per week) and high physical activity (>750 minutes per week) were associated with

graded reduction in mortality (hazard ratio 0.80, 95% CI: 0.74-0.87 and 0.65, 0.60-0.71; $p < 0.0001$), and major CVD (HR: 0.86, 0.78-0.93; $p < 0.001$), thus higher physical activity was associated with lower risk of CVD and mortality.

25% of adults in the North East do not achieve the minimum recommended levels of physical activity each week (at least 150-300 minutes of moderate-intensity aerobic physical activity) compared to 22% across England (Public Health England 2019). In China, the population in urban China was reported as having low physical activity during leisure time and a majority of the physical activity is work related. Moreover, with the increasing urbanization taking place in China, rates of physical activity could decline substantially over a relatively short period of time. This could have a negative impact on increasing risks of CVDs (Muntner, Gu et al. 2011). Recent study (Liu, Liu et al. 2020) also demonstrated that wide adoption of the physical activity recommendations would have considerable health impacts to the Chinese population and maintaining the recommended moderate to vigorous physical activity (MVPA) level could reduce the cardiovascular risk.

1.5.2 Tobacco smoking

Tobacco smoking increases the risk of contracting a wide range of chronic diseases, such as cardiovascular disease (Vidyasagan, Siddiqi et al. 2016). Stopping smoking at any age is beneficial compared with continuing to smoke (West 2017). It was estimated to be approximately 1 billion tobacco smokers worldwide, amounting to around 30% of men and 7% of women (Gowing, Ali et al. 2015).

In Britain, tobacco smoking is estimated to lead to the premature death of approximately 6 million individuals worldwide and 96,000 annually (Smoking and Health 2015). Tobacco smoking increases risk of CHD by elevating blood pressure and the tendency of blood clotting while lowering exercise tolerance and blood levels of HDL-C (Buttar, Li et al. 2005). Evidence suggested that even smokeless tobacco use leads to accelerated atherothrombosis similar to smoking (Gupta, Gupta et al. 2013). Meta-analysis (Vidyasagan, Siddiqi et al. 2016) observed that smokeless tobacco significantly increased risk of IHD deaths (odds ratios: 1.15, 95% CI: 1.01-1.30) and stroke deaths (OR:1.39, 95% CI: 1.29-1.49).

Passive smoking and second-hand smoking is also one of the harmful factors for cardiovascular health (Khoramdad, Vahedian-azimi et al. 2020). Meta-analysis of 18 studies including 10672 participants (Khoramdad, Vahedian-azimi et al. 2020) suggested that

passive smoking increase the risk of CVD incidence by 28% (adjusted RR = 1.28; 95% CI: 1.09, 1.50), where the highest risk was associated with those who were exposed to second-hand smoke at home and at work (adjusted RR = 1.41; 95% CI: 0.73, 2.70).

In China, for both gender in the same birth cohort (Wu, Zhu et al. 2020), the mortality rate of CVD attributable to smoking rapidly increased from 7.38 (95%CI: 6.34, 8.58) per 100,000 in the 30-34 age-group to 360.15 (95%CI: 338.97, 382.67) per 100,000 in the 75-79 age-group in China. Study (Jaana, Hanna et al. 2015) from Europe observed that smoking, as a strong independent risk factor of cardiovascular events and mortality, even at older age, increases cardiovascular mortality by over five years, and smoking cessation in any age groups has always been beneficial in reducing the risks of cardiovascular diseases.

1.5.3 Dietary patterns between Western and East Asian

Cardiovascular diseases are increasing mostly due to over utilization of fats from diets (Upadhyay 2015). Evaluating the full diversity of diet-related risk pathways, focusing on foods and overall diet patterns, rather than single isolated nutrients is of vital important (Mozaffarian 2016).

Typical western diet is likely to consist of high intakes of saturated fatty acid (SFA), trans-fat, salt, free sugar and excess calories. Conversely, intakes of polyunsaturated fatty acids (PUFAs), monounsaturated fatty acids (MUFAs) are insufficient (Anand, Hawkes et al. 2015). Resident food-environment characteristics, such as availability of grocery stores, convenience stores, and fast-food restaurants in the UK, are not consistently associated with diet quality or adiposity and could be related to determinants of CVD health (Anand, Hawkes et al. 2015).

Asian diets contain white rice as a main staple (Hu, Liu et al. 2011). White rice contributes approximately 30% of energy to diets in Asia. In China and Japan (Chan, Malik et al. 2009), a high consumption of foods with a high glycaemic index or load, such as white rice, doubles the risk of type 2 diabetes. Salt is another major part of Asian diets that is consumed in excess. The body needs 230-460 mg per day of sodium, and Asians average more than 4600 mg per day, which is excessive than the recommendation that adults should take no more than 6 grams of salt (2.4 grams of sodium) per day. Overconsumption of salt leads to high blood pressure, which is one of the main cardiovascular risk factors. In Japan and China, homemade meals with added soy sauce and salt, either while cooking or at the table, were the largest sources of sodium in the diet (Brown, Tzoulaki et al. 2009). Rural

communities in Asia often rely on salting as a method of food preservation, which may contribute to excessive salt intake (Chen, Gu et al. 2009).

Comparing with traditional East Asian eating habits without heavily sweetened drinks, sugar has become a growing problem in contemporary East Asian diets, which include a variety of teas, such as chai or bubble tea, that contain many calories and sugar in large serving sizes (Chan, Malik et al. 2009). Modern Asian foods also incorporate unhealthy fats such as lard into making Chinese desserts, such as Chinese rice pudding, and gutter oil, which leads to cheaper meal prices in private restaurants, food booth for many price-sensitive consumers after work. Trans fats are also increasingly part of packaged foods in East Asia due to their ability to prolong shelf life, yet there are no regulations requiring trans-fat content be disclosed to the public. It was reported that higher intake of trans fats is associated with poor cardiovascular health, and its risk factors such as weight gain as well as insulin resistance (Chan, Malik et al. 2009).

The traditional Southern River-style Jiangnan diet from China is reported to be similar to the Healthy Plate Recommendation by Harvard Nutrition (Swartzberg and Margen 2001) and the Jiangnan diet has gained in favour due to its apparent cardiometabolic disease - protection effect similar to that of the Mediterranean diet (Wang, Lin et al. 2020). Various southern diets, such as the Huaiyang, Aihui, and Zhejiang diets share common features, including high consumption of vegetables and fruits in season, freshwater fish and shrimp, and legumes; moderate consumption of wholegrain rice, plant oils (mainly rapeseed oil), and red meat; and relatively low consumption of salt, or millet wine. Steaming or boiling in clear soup and lukewarm-fire frying are the preferred cooking styles (Wang, Lin et al. 2020).

However, one study including 2659 subjects in Southeast China, Zhejiang province from 2010 to 2012 (Zhang, Wang et al. 2015) reported that the structure of the Chinese diet has been shifting away from the traditional diet toward high-fat, low-carbohydrate and low-fibre diets (Zhang, Wang et al. 2015). While the intake of carbohydrate in total energy in Chinese was lower than that in Japanese and American subjects, the Chinese food had lower daily intakes of fibre, calcium, phosphorus, potassium, selenium, vitamin A, vitamin B1, vitamin B2 and vitamin C, compared with the Japanese, American and Italian diets. However, intakes of sodium, iron, copper and vitamin E were higher among Chinese people relative to the people of other three countries (Zhang, Wang et al. 2015).

1.5.3.1 Dietary pattern in minority ethnic – East Asians (oriental ethnicity) in the UK

Dietary intake variations provide valuable clues when examined by migration status (Landman and Cruickshank 2001). Minority ethnic groups in the UK generally experience poorer health outcomes and suffer health inequalities compared with the mainstream white population, commonly attributed to the interaction between health behaviour patterns and genetic predisposition (Leung and Stanner 2011). In addition, few nutritional and dietary interventions have been carried out to explore the dietary needs of the Chinese previously.

The majority of the Chinese in the UK have family roots from Hong Kong and mainland China, with a smaller percentage from Malaysia, Vietnam, Singapore and Taiwan (Leung and Stanner 2011). The previous generation of Hong Kong citizens living in Britain during the 19th century inherited and adopted Asian-style diets incorporating unhealthy oils, such as those containing trans-fat and animal fat - salted butter, lard into cooking. Trans fats are also increasingly part of packaged foods in East Asia due to their ability to prolong shelf life, yet there are no laws requiring trans-fat content be disclosed to the public.

Evidence presented that traditional foods to shift after migration is via the process of acculturation (the assimilation of habits to that of the host country) (Leung and Stanner 2011). This change has been associated with poorer eating habits, especially in younger generations. Second generation offspring of former migrants are likely to adopt mainstream dietary patterns, with higher fat consumptions and lower vegetable, fruit and pulse intake compared with their first-generation parents. These changing dietary habits, alongside insufficient levels of physical activity, which is common among minority ethnic groups, might impact on the health of these minority ethnic groups (Leung and Stanner 2011). In addition, language and communication (e.g. poor interpretation services) and cultural differences (e.g. varying perspectives and beliefs on health) were identified as barriers to adopt healthier diets of minority ethnic groups such as Chinese immigrants (Leung and Stanner 2011).

1.5.3.2 British dietary pattern

Britain's unhealthy diet is associated with the concern of growing obesity and cardiovascular health issues such as heart attack, strokes since processed food such as crisps, chicken nuggets and poor-quality ready-made meals which are high in saturated fat, salt and sugars made up over half of the meals consumed in the average household (Mertens, Markey et al. 2017). While there has been reduction in the consumption of salt, sugar and red meat within the last ten years, there has been little change in the falling consumption of fruit,

vegetables and fibre, with high consumption of processed foods. In general, UK consumption still falls well short of nutritional guidelines (Mertens, Markey et al. 2017).

A study (Mertens, Markey et al. 2017) reported that British citizens with dietary pattern mainly characterised by high consumption of white bread, butter, lard, chips and sugar-sweetened beverages and lower intake of wholegrain bread, was associated with higher CVDs (HR 1.35; 95% CI: 1.10, 1.67) and stroke (HR 1.77; 95% CI: 1.18, 2.63) incidence.

Across the North East of England, evidence (Public Health England 2019) reported that approximately 25% of adults in the North East have no regular consumption of the recommended five portions of fruit and vegetables each day, although local authorities in the region are already using planning regulations to restrict the concentrations of fast-food takeaways where necessary.

1.5.4 Binge drinking

Modest to moderate amounts of alcohol consumption were reported to experience lower CVD rates compared with those who are abstinent or who consume heavily (Toma, Paré et al. 2017). However, binge drinking was defined as consuming over 14 units per week in both genders (a units of alcohol is 8 g or 10 ml of pure alcohol) and heavy episodic drinking are associated with an increased risk of mortality (NHS, 2020). Alcohol abuse was associated with an increased risk of incident atrial fibrillation (AF) (hazard ratio [HR]: 2.14; 95% confidence interval [CI]: 2.08 to 2.19; $p < 0.0001$), myocardial infarction (MI) (HR: 1.45; 95% CI: 1.40 to 1.51; $p < 0.0001$), and congestive heart failure (CHF) (HR: 2.34; 95% CI: 2.29 to 2.39; $p < 0.0001$) (Whitman, Agarwal et al. 2017).

British population cohort study (Bell, Daskalopoulou et al. 2017) suggested that heavy drinking (exceeding guidelines: UK weekly/daily of 21/3 and 14/2 units for men and women, respectively) conferred an increased risk of presenting with unheralded coronary death (hazard ratio:1.21, 95% confidence interval:1.08 to 1.35), heart failure (1.22, 1.08 to 1.37), ischaemic stroke (1.33, 1.09 to 1.63) and peripheral arterial disease (1.35; 1.23 to 1.48). In the North East of England, data suggested that 20% of adults in the North East regularly consume alcohol above the current low risk limit of 14 units a week but only one quarter of adults realise that alcohol can cause CVD (Public Health England 2019). In China, heavy drinkers experienced an approximately 1.3 fold and 1.7 fold greater risk for coronary heart disease and hypertension, respectively (OR: 1.252, 95% CI: 1.012 to 1.549; OR: 1.741, 95% CI: 1.519 to 1.994, respectively) compared with that of the non-drinking group (Li, Bai et al. 2016).

1.6 Traditional and novel risk factors for cardiovascular health

CVDs and atherosclerosis result from a combination of risk factors rather than just a single significant risk factor (Frostegård 2013). When multiple factors are at co-existence, the effects are augmented and exacerbated, which result in accelerated CVD development and increased mortality. Cardiovascular risk scores, such as the Framingham Risk Score is used to estimate the risks of developing CVDs in individuals before its clinical onset (Payne 2012).

CVD risk scores mainly incorporate the effects of well-established (or traditional) risk factors including hypercholesterolemia (dyslipidemia) (an abnormal amount of lipids) such as elevated LDL-C, especially with small LDL particle size, HDL-C less than 40mg/dL for men, less than 50 mg/dL for women; hypertension (greater than or equal to 140/90mm Hg or on antihypertensive medication), hyperglycemia, smoking, insulin resistance, diabetes, overweight or obesity, metabolic syndrome, inactivity, unhealthy diet, older age (45 years of age or older for men; 55 years or older for women), stress and a family history of premature CHD. Despite increasing evidence on the potential predictive power of other risk factors including high level of C-reactive protein (CRP), oxidation of LDL-C and high levels of triglycerides in the blood, these have not been integrated into these risk scores. Several emerging risk factors have shown their value in predicting CVDs and these are discussed below (Benjamin Emelia, Muntner et al. 2019).

1.6.1 Traditional CVDs risk factors

In CVD risk populations, rigorous control of blood cholesterol is recommended. A lipid profile, including total cholesterol, LDL-cholesterol, and HDL-cholesterol is a blood test which measures the concentrations of fats and cholesterol in the blood and they are also well-recognized risk factors for CVDs (**Table 1.1**) (Grundy Scott, Stone Neil et al. 2019).

Table 1.1 The optimal cholesterol level of healthy people.

	Total cholesterol	HDL cholesterol	LDL cholesterol	Triglycerides
Optimal or Desirable	Less than 200 (5.18 mmol/L)	Ideal is 60 or higher; 40 or higher for men and 50 or	Less than 100 (2.59 mmol/L); with CVD or	Less than 149; ideal is <100

	* the lower the better	higher for women is acceptable	diabetes; less than 70mg/dL	
Borderline to moderately elevated	200–239	n/a	130–159	150–199
High	240 or higher	60 or higher	160 or higher; 190 considered very high	200 or higher; 500 considered very high
Low	n/a	less than 40	n/a	n/a

All values are in mg/dl (milligrams per deciliter) and are based on fasting measurements.

1.6.1.1 Total cholesterol

In Western and Asian populations, epidemiological evidence showed the strong direct associations between total cholesterol and risks of CHD and ischemic stroke, both in middle and old age among both gender (Boehme, Esenwa et al. 2017). The American Heart Association (AHA) has defined untreated total cholesterol (TC) levels <200 mg/dl as one of the seven components of ideal cardiovascular health (Benjamin Emelia, Muntner et al. 2019). High total cholesterol was considered as the ninth and tenth-leading risk factor attributable to disability-adjusted life-years (DALYs) for men and women respectively in 2015 (Forouzanfar, Afshin et al. 2016).

According to one Korean cohort study (Yi, Shin et al. 2018), TC was inversely associated with stroke mortality (HR per 39 mg/dL; 95% CI = 0.80-0.95) in the range <200 mg/dL. TC was positively associated with stroke mortality in the upper range 200–349 mg/dL. The associations were similar in middle-aged (40-64 years) and elderly (≥65 years) adults and each 1 mmol/L (39 mg/dL) higher TC was associated with 11% higher mortality from stroke (95% CI: 2%-21%) in the elderly. Both middle-aged (39%) and elderly (23%) adults had higher ischemic stroke mortality associated with TC ≥240 mg/dL, compare to <200 mg/dL. One Chinese cohort study (Zhu, Lu et al. 2019) from Ningbo reported that individuals in the highest TC variability had 41% higher risk of CVD mortality (HR = 1.41, 95%CI: 1.10 to 1.81). Another meta-analysis suggested that raised TC is significantly stronger factor for CVD in men compared to women as the pooled relative risks (95% CI) for CHD associated with a 1 mmol/L increase in TC was 1.20 (1.16; 1.24) in women and 1.24 (1.20; 1.28) in men, resulting in a women-to-men ratio of RRs of 0.96 (0.93; 0.99) (Peters, Singhatheh et al. 2016).

1.6.1.2 Triglycerides

Triglyceride (TG) is the main form of dietary fat consisting of glycerol combined with three fatty acids or fats (saturated fat, unsaturated fat or both) combined with glycerol, a form of glucose. TG are the main source of energy supplying from diets and being made in liver (Heart UK 2019). Following food consumption, fats from foods are broken down in the liver into triglycerides. In addition, the liver converts excess calories (e.g., from drinking too much alcohol, sugar drinks, or eating too much fatty foods or meat) into triglycerides. These fatty triglycerides are released into blood circulation and are transported through the human body and utilized as energy or stored as fat. However, while TG plays an important role in metabolism, synthesis of hormones and building cells, high levels of TG in the bloodstream increase the risk of poor cardiovascular health (Alves-Bezerra and Cohen 2017).

Individuals with elevated triglyceride levels are at increased risk for cardiovascular disease (Ohmura 2019). Raised circulating TG levels were associated with increased CHD risk, adjustment for established coronary risk factors (Lee, Chang et al. 2017). A meta-analysis of 29 western studies showed that there was a significant association between TG values and CHD risk in Western populations (Odds Ratio (OR) 1.72; 95% confidence interval (CI), 1.56 to 1.90) (Sarwar, Danesh et al. 2007). In the Asia-Pacific region, participants with high triglyceride levels had a 70% (95% CI, 47 to 96) greater risk of CHD death compared with those with normal triglyceride level (Patel, Barzi et al. 2004). A Chinese cross-sectional survey (Ren, Ren et al. 2018) reported that a low level of triglyceride was associated with decreased risk of CVD (OR, 0.91, 95% CI: 0.88-0.93; OR, 0.94, 95% CI: 0.92-0.97) among patients with less 15 years of duration of diabetes but increased risk of CVD. Another study (Nichols, Philip et al. 2019) showed that the high TG group (Rate ratio (RR), 1.30; 95% CI: 1.08-1.58; P = 0.006) was 30% more likely to experience CVD such as non-fatal MI than normal TG group. The rate of experiencing CVD such as non-fatal stroke (RR:1.23, 1.01-1.49, P = 0.037) was 23% higher among high TG group than normal TG group.

1.6.1.3 LDL-cholesterol

Low density lipoprotein-cholesterol (LDL-C) is the major carriers of cholesterol in humans, and it is well recognized that hyperlipidemia, specifically elevated circulating LDL-C induces atherosclerosis and is a strong risk factor for heart diseases as excessive accumulation of LDLs at the arterial wall are the lipoproteins implicated in causing atherogenic plaque formation. The observational evidence supporting the importance of lifelong exposure to

elevated LDL levels as a cause of atherosclerotic cardiovascular disease (Gidding Samuel and Allen Norrina 2019).

Lower LDL-C levels were not only independently associated with a lower ischemic heart disease (IHD) mortality but also reduce cardiovascular risk independently of presence of inflammation (Storey, Staplin et al. 2018). On average, evidence showed that 1 mmol/L lower TC was associated with about a half (Hazard ratio (HR), 0.44; 95% CI: 0.42-0.48), a third (HR:0.66; CI:0.65-0.68), and a sixth (HR:0.83; CI:0.81-0.85) lower IHD mortality for both genders at ages 40-49, 50-69, and 70-89 years, respectively (Lewington, Whitlock et al. 2007). In addition, evidence suggested that a 10 mg/dL increase in LDL-C was associated with a 12% increase in CVD risk (Howard Barbara, Robbins David et al. 2000). Furthermore, in the study of 90000 participants in randomized trials of cholesterol-lowering treatment, a 1 mmol/l reduction in low-density lipoprotein cholesterol (LDL-C) was associated with a 23% reduction in coronary events, regardless of whether the initial diastolic blood pressure was above or below 90 mm Hg (Lewington and Clarke 2005).

1.6.1.4 HDL-cholesterol

High-density lipoprotein cholesterol (HDL-C) has more protein than LDL-C, allowing more cholesterol to be taken from the body's cells, resulting in greater transport and removal of cholesterol through the liver, where it is broken down and excreted in the bile (Feingold and Grunfeld 2000). HDL-C is one of the independent predictors of CVDs risk, lower levels of HDL-C increases risk of CVDs and higher levels have a cardio-protective effect (Mahdy Ali, Wonnerth et al. 2012). More importantly, HDL-C particles are presented to act as a protective factor against atherosclerosis via multiple biological mechanisms such as effluxing cellular cholesterol, diminishing cellular death, decreasing vascular constriction, reducing inflammatory response and protecting from pathological oxidation (Orozco-Beltran, Gil-Guillen et al. 2017).

The association between low HDL-C and atherosclerotic cardiovascular disease was shown by the Framingham study that HDL-C is a strong and independent cardiovascular risk factor for CVD and that the increase of HDL-C of 10 mg/L leads to a CHD risk reduction of 3% in women and by 2% in men (Mahdy Ali, Wonnerth et al. 2012). A study (Lewington, Whitlock et al. 2007) indicated that an increase of 0.33 mmol/L HDL-C was associated with a reduction of ischaemic heart disease (IHD) mortality by about a third within every age group and in both genders. Another study also showed that a 10 mg/dL decrease in HDL-C cholesterol associated with a 22% increase in CVD risk (Howard Barbara, Robbins David et al. 2000). Furthermore, HDL-C is reported to have a strong inherited basis with heritability

estimates of 40-60%, yet could be increased it to optimal levels by altering dietary patterns (Weissglas-Volkov and Pajukanta 2010).

1.6.1.5 Blood pressure

Hypertension, also known as high blood pressure is a long term condition in which the blood pressure in the arteries is persistently elevated (World Health Organization 2019). Each time the heart beats, the heart pumps blood into the vessels. Blood pressure is created by the force of blood pushing against the walls of blood vessels (arteries) as it is pumped by the heart. The higher the pressure the harder the heart has to pump (World Health Organization 2015). The excess strain and resulting damage from high blood pressure causes the coronary arteries serving the heart to slowly become narrowed from a buildup of fat, cholesterol and other substances that together are called plaque, in which chronic process is known as atherosclerosis (Rahman and Woollard 2017).

The ideal adult SBP is defined as within the range of 90 -139 mmHg, while the ideal adult DBP is defined as within the range of 60 - 89 mmHg, equal to or above 140/90 mmHg is indicative of hypertension (World Health Organization 2016). Evidence suggested that individuals with long term hypertension are at four times the risk of developing CVD (Zhou, Bentham et al. 2017). The WHO targets have called for a 25% reduction in the prevalence of high blood pressure by 2025 (World Health Organization 2016). Raised blood pressure affects 1.13 billion people worldwide (World Health Organization 2019) and long term elevated SBP is a leading global health risk (Forouzanfar, Liu et al. 2017). Long term high blood pressure is a major risk factor for heart failure, atrial fibrillation, chronic kidney disease, heart valve diseases, aortic syndromes, and dementia, in addition to coronary heart disease and stroke although high blood pressure does not cause any symptoms (Fuchs and Whelton 2020).

In the UK, nearly 30% of adults have high blood pressure (Townsend N, Bhatnagar P et al. 2015). In mainland China, hypertension prevalence was higher in the east region (32.6%) followed by the northeast region (31.8%) with inadequate awareness and treatment as 44.6% were aware of their condition, 35.2% were taking antihypertensive medication, and 11.2% achieved adequate BP control (Li, Lv et al. 2015). Additionally, statistic showed that systolic hypertension, with aging, tends to be a more significant issue as a result of progressive stiffening and loss of compliance of larger arteries as diastolic pressure, however, it is more likely to be elevated among people younger than 50 years (Saiz, Gorricho et al. 2018). After the age of 65, hypertension, considered a typical condition of aging, occurs in more than two thirds of individuals (Chobanian, Bakris et al. 2003). In

addition, evidence suggested that over 90% of individuals who are free of hypertension at 55 years of age are likely to develop it during their remaining lifespan (Lionakis, Mendrinou et al. 2012). In addition, seasonal variation of blood pressure is heightened in older adults is shown to be responsible for the greater cardiovascular disease mortality of elderly subjects during the winter (Kollias et al, 2020).

1.6.1.6 Ambulatory blood pressure

Ambulatory blood pressure monitoring (ABPM), refers to the records of BP readings across a 24-hour period which can be aggregated to yield overall 24-hour BP pattern or grouped to reflect daytime and nighttime BP pattern (Turner, Viera et al. 2015) compared with the traditional method of taking a BP reading under clinical setting and ABPM technically provides a more precise assessment of true BP than standard one-time measurement. Firstly, the sensitivity of pressure variability in ABPM depends on the factors such as circadian changes, BP variation with different environmental and emotional differences. Secondly, the advantage of ABPM is to rule out white-coat effect hypertension and identify masked hypertension, plus helps to reduce the number of possible false readings, along with the added benefit of understanding the dynamic variability of BP. Evidence (Dadlani, Madan et al. 2019) suggested that ABPM, involving serial assessment of upper arm pressures over 24 hours, usage of a small, automated, oscillometric device yields mean daily BP, revealing the time-integrated exposure of the brachial artery to BP throughout the day is a better predictor of major cardiovascular events than BP measurements at clinic settings as ABPM helps in reducing the number of possible false readings, along with the added benefit of understanding the dynamic variability of BP.

Higher 24-hour and nighttime BP readings were significantly associated with greater risks of death and a composite of cardiovascular outcomes and BP is normally highest during the day and lowest at night and absence of this rhythm is a predictor of cardiovascular morbidity and mortality. Evidence also showed that people with high 24-hour monitoring was better than one-off clinic reading had the highest risk of death (Hermida, Crespo et al. 2019).

From a Japanese study (Eguchi, Pickering et al. 2008), higher awake (SBP <135, 135-150, and >150 mm Hg) and sleep SBPs (<120, 120-135, and >135 mm Hg), predicted higher incidence of CVD in population with age 70.4 ± 9.9 years.

1.6.2 Novel risk factors for cardiovascular disease

Over the past few decades, apart from the traditional risk factors for cardiovascular diseases, epidemiological evidence emphasised the importance of processes, including endothelial dysfunction, the inflammatory processes involved in formation of plaque, and blood clotting and the tendency of oxidation in the subendothelial space (Badimon, Padró et al. 2012) (**Table 1.2**).

Table 1.2. Novel circulating biomarkers

	Parameter	Range	*Minimum detectable dose
1	sICAM-1	100 - 200 ng/ml	150 pg/ml
2	sVCAM-1	338.0 -1148.0 ng/ml	300 pg/ml
3	PAI-1	The upper limit at 50 mg/ml; 2-15 AU/ml	35 pg/ml
4	sP-selectin	Range from 19 - 521 ng/ml	20 pg/ml
5	sE-selectin	8.3 -116.9 ng/ml	30 pg/ml
6	IL-6	0 - 16.4 pg/ml	3 pg/ml
7	CRP	Below 3.0 mg/l.	34 pg/ml
8	Human SDC1 / Syndecan-1	Peaked at up to 500 ng/ml; 50–100 ng/ml	14 pg/ml

**The minimum detectable is according to the Sigma-Aldrich certificate of analysis/Protocol; *Keep in mind the normal reference range varies between labs.*

Inflammatory processes play a basal role in the pathogenesis of atherosclerotic vascular disease (Raggi, Genest et al. 2018), which is highlighted by the high cardiovascular risk of systemic inflammatory disorders (Thomas and Lip 2017).

Due to the complexities of CVD pathogenesis, no single biomarker is available to estimate absolute risk of future cardiovascular events. In addition, not all biomarkers are equal, and the functions of many biomarkers overlap. Some offer better prognostic information than others, and some are better suited to predict the pathogenesis of particular cardiovascular events (Buchan, Thomas et al. 2012). The identification of the most appropriate set of biomarkers can provide a detailed picture of the specific nature of the cardiovascular event (Buchan, Thomas et al. 2012). Novel biomarkers such as CRP, PAI-1, IL-6 have generated significant interest and tend to be widely investigated within the scientific community because they may provide additional means of improving CVD risk evaluation (Buchan, Thomas et al. 2012).

1.6.2.1 Pro-Inflammatory cytokines: C-reactive protein (CRP)

C-reactive protein (CRP), as an early marker of inflammation or infection, is an acute-phase inflammatory protein that elevates up to 1,000-fold at sites of infection in serum during inflammatory conditions such as rheumatoid arthritis and cardiovascular diseases (Emerging Risk Factors, Kaptoge et al. 2010). CRP plasma, is normally at concentrations of less than 10 mg/l in the blood (World Health Organization 2014). During infectious or inflammatory disease states, CRP levels rise rapidly within the first 6 to 8 hours and peak at levels of up to 350–400 mg/l after 48 hours (World Health Organization 2014) and increase from around 1 µg/ml to over 500 µg/ml within 24-72 h of severe tissue damage (Sproston and Ashworth 2018). CRP is synthesized primarily in liver hepatocytes but also by smooth muscle cells, macrophages, endothelial cells, lymphocytes, and adipocytes (Sproston and Ashworth 2018). Elevating circulating levels of CRP is associated with many non-communicable diseases and cardiovascular risk factors such as CHD, ischemic stroke, hypertension, metabolic syndrome, obesity, smoking, hypertension and peripheral artery disease (Shrivastava, Singh et al. 2015). Meta-analyses of CVD mortality risk has shown significant associations with CRP (HR 1.31, 95% CI: 1.02 to 1.68, p=0.033) (Barron, Lara et al. 2015).

Low plasma level of CRP (below 3.0 mg/l) is an indicator of health while high levels is an indication of inflammation in coronary heart disease (CHD). In healthy young adults, the median concentration of CRP is 0.8 mg/l, following an acute-phase stimulus, values may increase from less than 50 µg/l to more than 500 mg/l, that is 10,000-fold (Shrivastava, Singh et al. 2015).

The Cardiovascular Health Study Rural Health Promotion Project (Tracy, Lemaitre et al. 1997) with elderly participants of both gender identified CRP as a strong, independent risk factor for CHD. A large-scale prospective study documented a strong association between the predictive power of CRP and CHD risk with CRP levels being a more reliable biomarker of cardiovascular disease than LDL-C (Li, Sun et al. 2017). Meta-analysis showed that adjusted for all Framingham risk variables, the estimate of relative risk for incident CHD was 1.58 (95% CI, 1.37 to 1.83) for CRP levels greater than 3.0 mg/l compared with those with levels less than 1.0 mg/l (Buckley, Fu et al. 2009).

1.6.2.2 Pro-inflammatory cytokines - Interleukin-6 (IL-6)

Interleukin-6 (IL-6) is a pro-inflammatory cytokine and immunoregulating cytokine produced from adipose tissue (Buchan, Thomas et al. 2012). IL-6 acts on the liver to stimulate the

production of CRP and fibrinogen in an inflammatory response (Sproston and Ashworth 2018). IL-6 is elevated in patients with coronary heart disease and a marker of inflammation related to cardiovascular risk (Bacchioga, Bacchioga et al. 2017).

IL-6 levels increase with age and IL-6 levels are reported to be associated with higher mortality in nondisabled individuals more than age 65 from both cardiovascular and non-cardiovascular causes (Reiss, Siegart et al. 2017). A study which discovered within people with pre-existing stable coronary artery disease that higher IL-6 was associated with a poorer prognosis over an average follow-up of about six years as data showed that each 1 pg/ml increase in IL-6 was associated with a 1.70 pg/ml (95% CI:1.23-2.45) increased relative odds of subsequent myocardial infarction (MI) or sudden death (Fisman, Benderly et al. 2006). In a clinical study of 263 patients admitted to a hospital in Beijing, Chinese participants with ST-segment elevation MI, who were reported to have elevated circulating levels of IL-6 were correlated with higher cardiovascular mortality over 3 years of follow-up (Reiss, Siegart et al. 2017).

1.6.2.3 Plasminogen activator inhibitor (PAI-1)

Plasma concentrations of plasminogen activator inhibitor (PAI-1) is shown to be an potential independent risk factor for CVD (Ridker, Brown et al. 2004). Higher circulating PAI-1 levels are associated with increased risk of CVD and are associated with hypertension, diabetes mellitus, triglyceride levels, and homocysteine, and inversely related to HDL-C (Tofler, Massaro et al. 2016).

One study (Tofler, Massaro et al. 2016) showed that mean PAI-1 levels were $29.1 \pm SD19.2$ ng/ml for participants with incident CVD versus $22.1 \pm SD16.5$ ng/ml for those without incident CVDs. The same study (Tofler, Massaro et al. 2016) indicated that elevated PAI-1 reduce the capacity of the fibrinolytic system to prevent fibrin deposition in vessel walls and thrombus formation. Furthermore, as a marker of endothelial injury, PAI-1 could be an intermediary mechanism by which other risk factors injurious to the endothelium exert their effect (Tofler, Massaro et al. 2016). In another meta-analysis (Song, Burgess et al. 2017), it indicated a causal effect of elevated PAI-1 level on CHD risk. Moreover, a meta-analysis (Jung, Motazedian et al. 2018) also reported that elevated plasma PAI-1 levels 6.11 ng/ml (95% CI, 3.27-8.96) are associated with myocardial infarction, or cerebrovascular accident than those who are healthy.

1.6.2.4 Soluble cell adhesion molecules (sCAMs) – sICAM-1 and sVCAM-1

Adhesion molecules play an important role in platelet leukocyte interaction and leukocyte migration into the vessel wall, thus adhesion molecules are important players in the atherosclerosis process underlying cardiovascular diseases such as CHD (Kiechl, Paré et al. 2011). Evidence (Wang and Connolly 2010, Lam, Vijayan et al. 2015) showed that the levels of soluble adhesion molecules, including soluble vascular cellular adhesion molecule-1 (sVCAM-1), soluble intercellular adhesion molecule-1 (sICAM-1), and soluble E-selectin (sE-selectin), and soluble P-selectin (sP-selectin) were associated with increased risk of future death from CV causes among populations with CHD. Another previous study (Demerath, Towne et al. 2001) found a significant independent association between the concentrations of sICAM-1, sVCAM-1, e-selectin and cardiovascular risk factors including smoking, waist-hip ratio (WHR), blood pressure, high density lipoprotein cholesterol and total cholesterol in 592 healthy white adults aged 18-82 years. However, while E-selectin and P-selectin mediate transient rolling of leukocytes along the endothelium, sICAM-1 and sVCAM-1 mediate stronger attachment of leukocytes to the endothelium (Kunutsor, Bakker et al. 2017).

sICAM-1, as a general marker of a pro-inflammatory status, is expressed by endothelial cells in response to inflammatory cytokines and sICAM-1 participates in the adhesion of neutrophils to the endothelium, an crucial phase in the extravasation of neutrophils at the site of inflammation (Stoner, Lucero et al. 2013) and as suggested to be used as a marker of low-grade inflammation (Kilic, Findikoglu et al. 2015). Evidence showed that sICAM-1 appears consistently related to incident CAD as sICAM-1 correlates with acute phase reactants like CRP, and provides similar predictive information to CRP in settings of primary prevention in healthy populations (Lam, Vijayan et al. 2015). Previous data from a case-control study (Haim, Tanne et al. 2002) found that baseline serum concentrations of sICAM-1 were significantly higher in cases versus controls (375 vs. 350 ng/ml; $p < 0.05$). Each 100 ng/ml increase in sICAM-1 concentration was associated with 1.27 (95% confidence interval [CI]: 1.00 to 1.63) higher relative odds of coronary incidents (Haim, Tanne et al. 2002)

sVCAM-1 is expressed on both large and small vessels after the endothelial cells are stimulated by cytokines (Stoner, Lucero et al. 2013). sVCAM-1 is shown to be not appear as a risk factor in healthy individuals, but emerges as a strong risk predictor in patients experiencing pre-existing disease (Stoner, Lucero et al. 2013) because by contrast with sICAM-1, sVCAM-1 is not expressed in baseline conditions, but is rapidly induced by pro-atherosclerotic conditions in humans (Stoner, Lucero et al. 2013). Study (Tchalla, Wellenius

et al. 2015) found that elevated VCAM-1 level is associated with cardiovascular risk factors as sVCAM-1 concentration was 1195 ± 438 ng/mL in controlled hypertensives and 1250 ± 445 ng/mL in uncontrolled hypertensives ($p=0.008$). Another previous study from the Netherlands (Jager, van Hinsbergh et al. 2000) in a 7.4 year follow-up of 631 participants, demonstrated that elevated sVCAM-1 levels to be independently associated with cardiovascular mortality (relative risks per 100 ng/ml sVCAM-1 increase, 1.10 [1.05-1.15] after adjustment for age, sex, and glucose tolerance status) in subjects with type 2 diabetes.

1.6.2.5 Selectins - sP-Selectin and sE-Selectin

Soluble P-selectin (sP-selectin), is a member of the cellular adhesion molecule family, which also includes VCAM and ICAM-1 (Stoner, Lucero et al. 2013) while sE-selectin is a surface glycoprotein molecule expressed on endothelial cells (ECs) upon activation by cytokines and is therefore considered a superior marker of endothelial dysfunction compared to the other cell adhesion molecules, while sICAM-1 and sVCAM-1 are expressed on both ECs and leukocytes (Stoner, Lucero et al. 2013).

One study (Stoner, Lucero et al. 2013) reported that sP-selectin plays a key role in diseases associated with injury and arterial thrombosis. Increased expression of sP-selectin is observed in coronary artery disease, acute myocardial infarction, stroke, and peripheral artery diseases. Previous evidence (Blankenberg, Rupprecht Hans et al. 2001) indicated that increased levels of E-selectin have been observed in patients with acute coronary syndromes (ACS). A study from the UK (Krishnamoorthy, Khoo et al. 2013) found that higher levels of soluble E-selectin levels are associated with an increased risk of cardio adverse events (acute myocardial infarction, ischaemic stroke and all-cause mortality) as the highest tertile of E-seletion was associated with more adverse events [upper vs. lowest tertile, RR 3.7, 95% CI (2.51–5.31), $P < 0.001$; upper vs. middle tertile, RR 6.5, 95% CI (3.56–11.91), $P < 0.001$]. Another meta-analysis (Pletsch-Borba, Watzinger et al. 2019) found a consistent positive association between E-Selectin and cardiovascular risk factor such as T2D as E-Selectin was associated with higher T2D risk HR SD: 1.34 (95% CI: 1.16, 1.54; I² = 63%, n = 9 studies). Study (Pawelczyk, Kaczorowska et al. 2017) indicated that a strong correlation between higher sP-selectin concentration and enhanced LDL ($p = 0.001$), total cholesterol ($p = 0.02$) as the level of sP-selectin ($p < 0.001$) were significantly higher in groups of stroke patients compared with the controls. In addition, another study (Bielinski, Berardi et al. 2015) showed that a positive linear association between sP-selectin

levels and the rate of incidence of CHD (HR 1.63, 95% CI 1.15 to 2.30) after adjustments for traditional risk factors.

1.6.2.6 Human plasma syndecan-1 (SDC1)

Syndecan-1, as a transmembrane proteoglycan that exerts its functions mainly via its heparane sulfate chains, is a promising novel biomarker, correlated not only with the degree of cardiac fibrosis but also with the severity of liver fibrosis (Miftode, Şerban et al. 2019). From one recent Chinese study (Liu, Wang et al. 2019) in heart failure (HF) patients, higher plasma syndecan-1 levels was reported to be an independent risk factor for the incidence of adverse cardiovascular events.

A previous study observed that the particularity of the syndecan-1 molecule, related to lipid metabolism, showed that membrane syndecan-1 can mediate the binding and uptake of the very low-density lipoprotein cholesterol (VLDL-C) (Deng, Foley et al. 2012). A later study (Tromp, Van Der Pol et al. 2014) indicated that plasma syndecan-1 levels in 567 patients with chronic heart failure twice the cut-off value was associated with an increased risk of all-cause mortality after 18 months of follow-up. Similarly, study (Neves, Meneses et al. 2015), investigating 201 patients with acute decompensated heart failure found that the level of syndecan-1 was associated with in-hospital mortality rates and also showed a significant separation of 6-month survival curves for patients with low and high levels. A recent review (Freitas, Lima et al. 2020) indicated that increased expression of syndecan-1 is observed in myocardial infarction (MI) and protects against an exacerbated inflammatory response.

1.6.3 Other determinants on cardiovascular health

Healthy dietary patterns and healthy lifestyle are seen as modifiable weapons in the fight against cardiovascular diseases and the most effective means in which to prevent the occurrence of CVDs in society in spite of the fact that biological determinant of cardiovascular health has been considered irreversible (Kreatsoulas and Anand 2010, Kumar 2017).

Apart from the behavioral factors as well as traditional biomarkers shown above, it is crucial to address the socio-economic factors such as food, housing, financial instability, and healthcare access that promote the development of risk factors for cardiovascular diseases (Parekh, Desai et al. 2020). The amount of the population (8.8%) who had CVDs, compared

to non-CVDs respondents, food insecurity (27.5% vs. 21.2%), financial insecurity (18.2% vs. 8.32%) were significantly higher among those with CVDs. While financial insecurity showed 2 times higher odds of CVDs (AOR 2.14 [1.73–2.66]) compared to non-CVDs (Parekh, Desai et al. 2020).

1.6.3.1 Socioeconomic determinants of cardiovascular health

Socioeconomic status measures such as education, income and occupation have been explored extensively associated with cardiovascular health and low social economic conditions is associated with a greater prevalence of CVD risk factors (Havranek, Mujahid et al. 2015).

Lower levels of educational attainment are associated with a higher prevalence of cardiovascular risk factors, higher incidence of cardiovascular events, and higher cardiovascular mortality, independent of sociodemographic factors (Rosengren, Smyth et al. 2019). A study by (Mackenbach, Cavelaars et al. 2000) examined the higher mortality among individuals with lower education in the United States and 11 Western European countries and the result reported that a widening education-based difference in cardiovascular death was responsible for 17.4% of the overall gap in life expectancy, second only to cancer.

The burden of CVDs is rising disproportionately among lower income countries and populations and CVD was more prevalent in lower income households (Havranek, Mujahid et al. 2015). Evidence (WHO 2017) showed that citizens who live in low- and middle-income countries are likely to experience less or even zero benefit of integrated primary health care programmes for early detection and treatment of people with risk factors compared to citizens in high-income countries and over three quarters of CVD deaths take place in low- and middle-income countries. A large study (Schultz, Kelli et al. 2018) in the United States and Finland found an increased risk of nonfatal myocardial infarction and sudden cardiac death in the low-income cohorts that persisted after adjusting for smoking and alcohol consumption (Schultz, Kelli et al. 2018).

Unemployment has been associated with increased risk of CVD (Schultz, Kelli et al. 2018). Higher status occupations were associated with less hypertension and protective service workers such as firefighters had the lowest rates of treatment for established hypertension. Epidemiological studies of unemployment and health are particularly difficult because of potential “effect-cause” relationships, in which unemployment is a consequence of poor

health rather than the reverse, and because of confounding by factors such as low educational attainment that might predict both unemployment and poor health. Nonetheless, the preponderance of evidence supports the position that job loss leads to illness (Havranek Edward, Mujahid Mahasin et al. 2015).

Linguistic and cultural differences contribute to poorer cardiovascular health in disadvantaged groups (Havranek Edward, Mujahid Mahasin et al. 2015). Culture is as commonly used as a system of beliefs and behaviours characteristic of a definable group that is transmitted without biological inheritance. Misunderstandings rooted in differing cultural perceptions of disease can play an important causal role in health disparities (Havranek Edward, Mujahid Mahasin et al. 2015).

1.7 Dietary intervention – the Mediterranean diet to improve cardiovascular health

Dietary interventions delivering comprehensive management towards improving the risk factors for CVD are essential (Li and Ge 2014). Promising data emerged from well-designed randomized trials and meta-analyses, and unprecedented progress was made in the last few decades in discovering novel effective dietary strategies for CVD prevention and treatment (Ravera, Carubelli et al. 2016). An Asian population cohort (Leu, Chung et al. 2019) indicated that a healthy diet pattern, such as the Mediterranean diet, could possibly modify and reduce the genetic risk of cardiovascular diseases such as myocardial infarction and the impact of dietary pattern was analysed based on the adherence to the Mediterranean Diet Score.

A study (Scholl 2012) reported that the low-fat-high-carbohydrate (LFHC) diet proposed for CVD prevention was recommended to individuals with overweight, metabolic syndrome, and type 2 diabetes, although the main emphasis of LFHC diet was lowering of TC and LDL-C, irrespective of the known effects of such a high glycaemic load on HDL-C and glucose metabolism. There are no studies showing that the whole of “The Japan Diet” is more beneficial than the Western diet (Teramoto 2017). Insufficient epidemiological studies allow the meta-analysis of researching on “The Japan Diet” (Liyanage, Ninomiya et al. 2016) or the Dietary Approaches to Stop Hypertension (DASH) diet (Siervo, Lara et al. 2015). The DASH Diet is in fact a Mediterranean-style diet with 10 portions of fruits and vegetables per day, more dairy products, low sodium content, and less sugar and starch, discourages sugar-sweetened foods and beverages (Scholl 2012). However, the DASH trials were not designed to evaluate the impact of DASH diet on CVD clinical events. Further improvements in cardiovascular risk factors were seen when part of the carbohydrate in DASH was

replaced with either monounsaturated fat or protein (Appel, Sacks et al. 2005). Furthermore, DASH diet has reported a poor compliance and being challenging to be maintained as salt intake is required to be 2,300 mg/day and potentially as low as 1,500 milligrams daily (Kwan, Wong et al. 2013). The Portfolio Diet, which includes 30 g/day of nuts, 20 g/day of viscous fibre (i.e., oats, barley, legumes), 80 g/day of vegetable protein (soy, beans, chickpeas, lentils), and 2 g/day of plant sterols (plant sterol margarine) from plant foods lowers LDL-C by up to 30% within four weeks, similar to statins. Reductions of 13%-23% are maintained when this diet was extended to 12 months. However, no RCT has evaluated the impact of this diet on CVD clinical events (Anand, Hawkes et al. 2015).

Since cardiovascular risk factors such as diabetes, all of which have been reported to be associated with worse outcomes of the coronavirus disease 2019 (COVID-19), the Mediterranean diet, as highly recommended healthy dietary pattern, prevents diabetes from improving glucose control in diabetic patients, exerts anti-inflammatory and immunomodulatory effects was reported to be a promising and relatively obtainable approach in daily life to attenuate the severity of COVID-19 infection (Angeliki et al, 2020; Maria Ida et al, 2020).

In addition, according to Ana et al, (2021), adherence to the Mediterranean-style diet play an essential role in the establishment of proper dietary pattern in lockdown situations in Spain, particularly among older population since Mediterranean-style diet was evidently shown to contribute to the prevention of weight gain, BMI due to high levels of isolation, prevention of neurodegenerative illness with anxiety, depression and also to the modification of the immune and inflammatory response (Ana et al., 2020).

Strong evidence from prospective cohort studies and randomized trials shows reduced CHD with the consumption of a Mediterranean diet (MD) (Mente, Koning et al. 2009). Therefore, MD is widely believed as a cardio-friendly determinant of the wide difference in CVD prevalence between Mediterranean and the Western populations since the traditional dietary MD pattern followed by Mediterranean populations in the early 1960s (Ros, Martínez-González et al. 2014). Plus, due to the ingredients in MD, MD is believed to have beneficial impacts on the majority of cardiovascular risk factors, such as body mass index, waist circumference, blood lipids, blood pressure, inflammatory markers and adhesion molecules, and diabetes. Evidence (Tuttolomondo, Simonetta et al. 2019) reported that these advantages of the Mediterranean diet are maintained in comparison of a low-fat diet.

MD is characterized by the following: 1) abundant use of olive oil; 2) high consumption of plant foods (fruits, vegetables, legumes, cereals, nuts, and seeds); 3) frequent but moderate

intake of wine (especially red wine) with meals; 4) moderate consumption of fish, seafood, fermented dairy products (yogurt and cheese), poultry, and eggs; and 5) low consumption of red and processed meat and sweets (Ros, Martínez-González et al. 2014, Ravera, Carubelli et al. 2016). In the PREDIMED study, MD supplemented with EVOO (1 liter/week), compared to the control group (low-fat diet), was able to significantly reduce proinflammatory cytokines (IL-6, sP-selectin, sVCAM-1 and sICAM-1) in subjects at high cardiovascular risk (Casas, Sacanella et al. 2014).

1.7.1 Extra virgin olive oil in the Mediterranean diet

Extra virgin olive oil (EVOO) is responsible for a large proportion of cardio benefits associated with the Mediterranean diet as it is a fundamental ingredient of this diet (Mazzocchi, Leone et al. 2019). EVOO is associated with reduced risks of cardiovascular disease and mortality in individuals at high cardiovascular risk in the PREDIMED study (Guasch-Ferré, Hu et al. 2014). The consumption of 50 ml/day of EVOO may reduce the possibility of developing coronary artery disease (CAD) by 37%, and the incidence of major cardiovascular events by 30% (Estruch, Ros et al. 2013). Recent studies (Nocella, Cammisotto et al. 2018, Zamora-Zamora, Martínez-Galiano et al. 2018, George, Marshall et al. 2019, Schwingshackl, Krause et al. 2019, Tsartsou, Proutsos et al. 2019) illustrated the healthy benefits of olive oil intake as well as the benefits of nutraceutical properties in olive oil to cardiovascular health.

1.7.1.1 Composition of extra virgin olive oil (EVOO)

EVOO is mainly composed of triglycerides (97%-99%) and minor compounds (1%-3%), which are the principal components responsible for its biological properties and sensory attributes (Jimenez-Lopez, Carpena et al. 2020).

Depending on its origin and extraction process, in terms of the lipid content of EVOO, EVOO predominantly has a high content of monounsaturated fatty acids (MUFAs), especially with oleic acid being the fraction representing 55%-83%, followed by the minor fractions of polyunsaturated fatty acids (PUFAs), representing 4% to 20% such as linoleic acid (LA) (the shortest-chain of the common omega-6 fatty acids) and alpha-linolenic acids (ALA) (a type of omega-3 fatty acid). Saturated fatty acids (SFA), representing 8% to 14%, such as palmitic and stearic acids. This lipid profile has been linked to protective effects on coronary and inflammatory disorders but also as anti-thrombotic and regulators of blood pressure

(Jimenez-Lopez, Carpena et al. 2020). Regarding bioactive compounds, their main representatives are the same of oil in general, namely minor phenolic compounds such as hydroxytyrosol and derivatives (oleuropein and tyrosol), tocopherols but also other compounds as hydrocarbons (i.e., squalene) (Lombardo, Grasso et al. 2018).

To present antioxidant activity in body, minor components preserving other components are presents, such as vitamin E; Among the antioxidants present in EVOO is hydroxytyrosol. This compound exhibits anti-inflammatory and anti-teratogenic activity, improving the lipid profile, and reducing oxidative stress and the activation of inflammatory cells. Oleuropein, another antioxidant found in olive oil has also been associated with the improvement of anti-inflammatory parameters.

Bioactive components of EVOO demonstrated improvements in inflammatory status, oxidative stress, and endothelial dysfunction (Wongwarawipat, Papageorgiou et al. 2018). Besides that, during recent years, unsaponifiable fraction (minor components) constitutes 1%-2% of the total content of EVOO, containing aliphatic and triterpenic alcohols, sterols, hydrocarbons, tocopherols, β -carotene, phytosterols, pigments and volatile compounds also received attention owing to its health benefits, such as blood cholesterol control (Kyçyk, Aguilera et al. 2016) (**Table 1.3**).

Table 1.3. Nutritional components present in extra virgin olive oil.

Component	Amount Per 100 g Olive Oil
Energy	884 kcal/3699 kJ
Carbohydrates, fibre	0-0.2 g
Protein	0
Fat	100 g
• saturated FA	• 14 g
• mono-unsaturated FA	• 73 g (up to 73% of RDA)
• poly-unsaturated FA	• 13 g
Cholesterol	0
Vitamin A	0-157 μ g
Vitamin E	0-37 mg (up to 72–96% RDA)
Vitamin K	55-60 μ g (up to 50–75% RDA)
Sodium	1-2 mg
Potassium	0-1 mg
Calcium	0-1 mg
Magnesium	0-1 mg

Phosphor	0-2 mg
Iron	100-560 µg (up to 7% RDA)
Zinc	10-60 µg
Copper	0-70 µg
Chlorophyll	0.5-1.6 mg
(Poly)phenols and phenolic compounds (n = 36), e.g.,	28-221 mg
<ul style="list-style-type: none"> • Oleuropein • Tyrosol • Hydroxytyrosol • Oleocanthal 	

Values might differ considerably according to olive cultivar, climate conditions, and production process of oil (Rizwan, Benincasa et al. 2018).

1.7.1.2 The effects of composition of extra virgin olive oil on cardiovascular health

Polyphenols are the most abundant dietary antioxidants present in EVOO, which possess a wide range of health effects in the prevention of CVD (Mozaffarian and Wu 2018). PUFAs, as omega-3 fatty acid, α -linolenic acid (ALA), eicosapentaenoic acid (EPA), and docosahexaenoic acid (DHA), were reported as potential anti-atherogenic agents for the atherosclerotic process (Shahidi and Ambigaipalan 2018). Mechanisms, through which they might reduce CV risk, include improvements in the lipid and lipoprotein profile, oxidation, thrombosis, endothelial function, blood pressure, plaque stability, CV mortality, platelet aggregation, modulating concentration or expression of pro-inflammatory markers (adhesion molecules, cytokines, etc.), and immune cells (Shahidi and Ambigaipalan 2018). Meta-analysis of 16 randomized placebo-controlled trials in 901 participants reported that omega-3 PUFA intake (0.45-4.5 g/day, for 56 days) increased FMD by 2.30% (95% CI: 0.89, 3.72%, $p = 0.001$) compared with the placebo group (Wang, Liang et al. 2012).

Olive oil phenolic compounds impact a huge beneficial effect on inflammation (Souza, Marcadenti et al. 2017) because the concentration of phenolic compounds in EVOO (551.4 mg/kg; ranging from 50-800 mg/kg) is higher than regular olive oil (206.7 mg/kg), and refined olive oil (198-62.0 mg/kg), and the concentration of phenolic compounds in EVOO is influenced by the extraction procedure of the oil. EVOO is obtained by mechanical processes, while refined olive oil (ROO) is subjected to both physical and chemical procedures. Even If ROO presents a similar composition of fatty acids, due to the low

phenolic content, it does not bring the same beneficial effects when compared with EVOO (Souza, Marcadenti et al. 2017).

Phenols such as hydroxytyrosol (HT) and derivatives (oleuropein complex and tyrosol) are primarily responsible for the beneficial effects of EVOO in the prevention and progression of atherosclerosis, by improving endothelial function (Moreno-Luna, Muñoz-Hernandez et al. 2012), antioxidant effect (Del Carlo, Sacchetti et al. 2004), the high density lipoprotein function (Hernández, Fernández-Castillejo et al. 2014) and reducing the concentration and the atherogenicity of the low density lipoprotein (Fernández-Castillejo, Valls et al. 2016). To ensure the cardiovascular benefits of olive oil, the European Food Safety Authority recommends the daily intake of 5 mg of HT or its derivatives, which can be obtained by the daily consumption of at least 20 g of EVOO.

The ratio of unsaponifiable matter in the olive oil is about 1 to 2% and much of this fraction is represented by phytosterols, which are recognized by their biological effects in EVOO (Kyçyk, Aguilera et al. 2016). Evidence (Gylling, Plat et al. 2014) suggested that a daily dose of 2–3 g of plant sterols or phytosterols is associated with an LDL-C reduction of around 6–15% of the total concentration. These reductions were also observed in a meta-analysis (Demonty, Ras et al. 2009) where after administering a daily dose of 2.15 g of phytosterols, LDL-C was reduced by 8.8%. In another meta-analysis (Rocha, Ras et al. 2016) of 20 RCTs with 1308 participants, the effect of phytosterols intake on pro-inflammatory markers observed that the significant reductions of CRP levels (–0.10 mg/dL) after plant sterols' intake.

1.7.2 Nuts in the Mediterranean diet

The Mediterranean diet supplemented with nuts provides primary cardiovascular disease prevention benefits (Widmer et al., 2014). A Mediterranean diet supplemented with 30 g of mixed nuts (walnuts, almonds and hazelnuts) per day also showed beneficial effects on the lipid profile compared with advice on a low-fat diet in diabetic and non-diabetic participants in the PREDIMED study, a randomized trial of dietary intervention for the primary prevention of cardiovascular disease (Ramon et al, 2006).

Meta-analysis of prospective cohort studies (Liu et al, 2020) showed that per 0.5 serving/day increase in total nut consumption was associated with lower risk of cardiovascular disease (relative risk [RR], 0.92; 95% CI, 0.86–0.98), coronary heart disease (RR, 0.94; 95% CI, 0.89–0.99), and stroke (RR, 0.89; 95% CI, 0.83–0.95).

A meta-analysis of observational studies (Kelly 2010) reported an inverse relationship between nut consumption and cardiovascular disease, with an approximately 40% decrease in the incidence of primary cardiovascular disease with consumption of at least four nut servings per week and up to 10% reduction with a single serving per week.

Randomized controlled trial with walnuts (Ma et al., 2010) almonds (Jenkins et al, 2008), hazelnuts (Mercanligil et al, 2006), pistachios (Alaupovic et al, 2008), macadamias (Griel et al.,2008), and peanuts (Lokko et al., 2007) showed LDL-cholesterol reductions ranging from 4% to 11% versus comparator diets, confirming the cholesterol-lowering efficacy of various nut types.

1.7.2.1 Nutrient composition of nuts

Nuts are nutrient dense foods with complex matrices rich in unsaturated fatty and other bioactive compounds, such as high-quality vegetable protein, fiber, minerals, tocopherols, phytosterols, and phenolic compounds (Ros 2010).

With the exception of chestnuts, which contain little fat, nuts have a high total fat content, ranging from 46% in cashews and pistachios to 76% in macadamia nuts, and they provide 20 to 30 kJ/g (Ros and Mataix, 2006). The fatty acid composition of nuts is beneficial because the saturated fatty acid (SFA) content is low (4 -16%) and almost half of the total fat content is made up of unsaturated fat, monounsaturated fatty acids MUFA (oleic acid) in large majority of nuts, similar proportions of MUFA and polyunsaturated fatty acids (PUFA), mostly linoleic acid, in Brazil nuts, a predominance of PUFA over MUFA in pine nuts, and mostly PUFA, both linoleic acid and α -linolenic acid (ALA), the plant omega-3 fatty acid, in walnuts (Ros and Mataix, 2006). In addition, nuts are rich in arginine, an amino acid needed to make a molecule called nitric oxide that relaxes constricted blood vessels and eases blood flow. They also contain vitamin E, folate, potassium, fiber, and other healthful nutrients (Ros and Mataix, 2006). Among the constituents of nuts there are significant amounts of essential micronutrients such as sizeable amounts of folate that are also associated with an improved health status when consumed at doses beyond those necessary to prevent deficiency states (**Table 1.4**).

1.7.2.2 The effects of composition of nuts on cardiovascular health

Nuts are rich sources of antioxidant vitamins (e.g., tocopherols) and phenolic compounds, necessary to protect the germ from oxidative stress and preserve the reproductive potential of the seed, but also bioavailable after consumption and capable of providing a significant antioxidant load (Blomhoff et al., 2006).

Walnuts are considered to be more anti-inflammatory than other nuts as walnuts are the only nuts that contain substantial amounts of ALA, which is described as one of the more anti-inflammatory fatty acids. Walnuts are also particularly rich in the phenolic compound ellagic acid, which evokes a reduction in concentrations of other inflammatory biomarkers studies (Karlsson et al, 2010; Papoutsi et al., 2008). Almonds in particular are especially rich in α -tocopherol, while walnuts contain significant amounts of its isomer γ -tocopherol, which has been investigated much less than α -tocopherol, but is increasingly recognized as a relevant antiatherogenic molecule (Wagner et al, 2004). Remarkably, in all nuts most of the antioxidants are located in the pellicle or outer soft shell, as shown for almonds (Chen et al, 2005) and peanuts (Lou et al, 2004), and 50% or more of them are lost when the skin is removed (Blomhoff et al, 2006).

Nuts are cholesterol-free, but their fatty fraction contains sizeable amounts of chemically related noncholesterol sterols belonging to a heterogeneous group of compounds known as plant sterols or phytosterols (Segura et al, 2006). Phytosterols interfere with cholesterol absorption and thus help lower blood cholesterol when present in sufficient amounts in the intestinal lumen. The mechanism of action of phytosterols has been linked to their hydrophobicity, which is higher than cholesterol because of a bulkier hydrocarbon molecule and entails a higher affinity for micelles than has cholesterol.

Compared to other common foods, nuts have an optimal nutritional density with respect to healthy minerals, such as calcium, magnesium, and potassium. Like that of most vegetables, the sodium content of raw or roasted but otherwise unprocessed nuts is very low, ranging from undetectable in hazelnuts to 18 mg/100 g in peanuts (Segura et al, 2006). In addition, A high intake of calcium, magnesium and potassium, together with a low sodium intake, is associated with protection against bone demineralization, arterial hypertension, insulin resistance, and overall cardiovascular risk (Cordain et al, 2005).

As a result, the macronutrient, micronutrient and non-nutrient components of nuts shown in **Table 1.4** have been documented to contribute to a reduced risk of CHD and related metabolic disturbances.

Table 1.4. Average nutrient composition of nuts (per 100 g).

Nuts type	Energy (kJ)	Fat (g)	SFA (g)	MUFA (g)	PUFA (g)	LA (g)	ALA (g)	Protein (g)	Fiber (g)	Folate (µg)	PS (mg)
Almonds	2418	50.6	3.9	32.2	12.2	12.2	0.00	21.3	8.8	29	120
Brazil nuts (dried)	2743	66.4	15.1	24.5	20.6	20.5	0.05	14.3	8.5	22	NR
Cashews	2314	46.4	9.2	27.3	7.8	7.7	0.15	18.2	5.9	25	158
Hazelnuts	2629	60.8	4.5	45.7	7.9	7.8	0.09	15.0	10.4	113	96
Macadamia nuts	3004	75.8	12.1	58.9	1.5	1.3	0.21	7.9	6.0	11	116
Peanuts	2220	49.2	6.8	24.4	15.6	15.6	0.00	25.8	8.5	145	220
Pecans	2889	72.0	6.2	40.8	21.6	20.6	1.00	9.2	8.4	22	102
Pine nuts (dried)	2816	68.4	4.9	18.8	34.1	33.2	0.16	13.7	3.7	34	141
Pistachios	2332	44.4	5.4	23.3	13.5	13.2	0.25	20.6	9.0	51	214
Walnuts	2738	65.2	6.1	8.9	47.2	38.1	9.08	15.2	6.4	98	72

Data for raw nuts, except where specified. SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; LA, linoleic acid; ALA, α-linolenic acid; PS, plant sterols; NR, not reported.

Source: US Department of Agriculture Nutrient Data Base.

1.8 Acceptability of the Mediterranean diet in the UK and China

A healthy diet is a significant adaptable determinant of vascular healthy ageing (Marsman, Belsky et al. 2018). Worldwide, humans have scrutinised micro and macronutrients in order to find the optimal dietary balance that minimise the insidious effects of ageing for years (Mooney and Mc Auley 2016). As the Mediterranean diet has been widely accepted as being associated with a lower risk of chronic diseases such as cardiovascular mortality (Estruch, Ros et al. 2018, Rosato, Temple et al. 2019), coronary diseases (Dontas, Zerefos et al. 2007), obesity, type 2 diabetes, mellitus and metabolic syndrome in adults (Kastorini, Milionis et al. 2011, Huo, Du et al. 2015), the protective effect of the Mediterranean diet as an important lifestyle strategy for reducing the incidence of cardiovascular disease and promoting longevity has been highly recommended (Sofi 2009, Buckland, Agudo et al.

2011). Nonetheless, the feasibility to implement a Mediterranean diet in non-Mediterranean regions such as China or UK remains uncertain and requires further exploration.

While the Mediterranean-type diet is reported to be comparably easily transposable to non-Mediterranean regions (Speed 2004), the Mediterranean diet is difficult to be widely adopted since it is not only a list of foods, it encompasses a social, cultural, and agricultural way of life. Cooking and preparing foods brings conviviality and sharing of food for festivities, celebrations, social, and religious traditions and with family and friends (Lăcătușu, Grigorescu et al. 2019). It embodies frugality, biodiversity, seasonality, minimizing waste and long-term sustainability. In addition, current Western countries like the UK are among the highest global consumers of meat.

In the UK, the typical diet consumed by UK adults is considerably different to the Mediterranean diet, being low in fruit and vegetables, legumes, oily fish and wholegrains, and high in saturated fat and sugar (Hoffman and Gerber 2013). Barriers such as perceived difficulty of living in a colder climate, body weight, cultural difference, and lower income was reported to limit Mediterranean diet adoption by adults at high risk of cardiovascular disease from a Northern European population (Moore, McEvoy et al. 2018). In addition, a survey study (Francis, Young et al. 2018) from Northeast England including 554 individuals (86.9 % female) also presented the result that the adherence to the overall MD is currently low, however, the MD guidelines are seen as acceptable by the local population including overweight and obese individuals from the Northeast England.

China was reported to have a comparable higher acceptability of Mediterranean diet than the UK during previous years (Woo, Woo et al. 2001). Questionnaire research (Woo, Woo et al. 2001) suggested that in Hong Kong and China, more women in the middle age group (35-54y) had a high healthy Mediterranean score than other age groups, and overall more female subjects had high scores than men. After investigating the dietary patterns of a Chinese population living in Hong Kong, Guangdong province in Southern China, Sydney and San Francisco (Woo, Woo et al. 2001), Guangdong province had the highest number of subjects with high healthy Mediterranean score. The large majority of the Chinese population who consume a diet comparable to the traditional Mediterranean diet, whether in China or in Western countries are likely to have health benefits concerning coronary heart disease and survival. The study reported that most Chinese participants were aged 40 years and over and it was believed that there is less geographical variation in dietary habits among the older population, reflecting a tradition of remaining more traditional Chinese diet patterns rather than adopting 'Westernized' diets than younger generation (Woo, Woo et al. 2001).

Britain was reported to have a promising tendency for adopting MD as one randomized controlled trial conducted in Britain suggested that the acceptability of the Mediterranean diet as a healthy eating guide among healthy British older adults is likely to be encouragingly feasible (Lara, Turbett et al. 2015) and MD guidelines are likely to be accepted by the British population including overweight and obese young individuals. Furthermore, MD is also recommended to be promoted in work-place settings for working employees in the England (Papadaki, Wood et al. 2015).

Research questions raised from the present Chapter 1:

The work described in this chapter raised the following questions:

1. What is the acceptability and frequency consumption of nuts and emphasis in olive oil consumption among Caucasians (mostly British living in Northeast England) and East Asians (mostly Chinese living in Newcastle upon Tyne of Northeast England)?
2. What is the state of the evidence from interventional studies assessing the impact of foods including nuts as well as olive oil consumption on CVD risk factors?
3. Is the effect of these interventions on cardiovascular risk factors similar between different ethnic population namely Caucasians and East Asians living in the Northeast England?

These questions were addressed in **Chapter 2** to **Chapter 5**. In the survey chapter (**Chapter 2**), nuts and olive oil consumption among 142 East Asians and 142 Caucasians and their association with PREDIMED score, perceived barriers and acceptability of the Mediterranean diet will be explored and presented. Systematic and quantitative evaluation of the evidence from randomized controlled trials, is presented in **Chapter 3** and **Chapter 4**, reporting the associations between olive oil and nuts intake and risk of CVDs. Overall, the combination of the findings from the survey and systematic reviews will determine the dietary intervention to be tested in the further clinical trial.

CHAPTER 2

Nuts, olive oil consumption in Mediterranean diet among East Asians and Caucasians and their association with MD acceptability, PREDIMED score and perceived barriers to healthy eating: an online survey

Abstract

Objectives.

PREDIMED studies indicate that the Mediterranean diet (MD) supplemented with nuts and olive oil resulted in a reduction of around 28% and 30% cardiovascular risks respectively around the Mediterranean basin but provided unclear effects on Asians and British populations. Therefore, this study aims to identify different degree of acceptability, feasibility and consumption of walnuts and olive oil between East Asians (mostly Chinese) and Caucasians (mostly British) to inform the design of nutritional interventions to improve cardiovascular health for both ethnicities in the near future.

Study design.

From August 2017 to April 2018, responses from East Asians and Caucasians were collected via an anonymous online Survey named "Public Opinions of the Mediterranean Diet". Data analyses included two-step cluster analysis, t-tests and Chi-square tests analysing data. Results from 142 Caucasians (87.3% female; mean age: 32.8±SD12.2; BMI: 22.8±SD2.8) resident in the North East of England and 142 East Asians (78.2% female; mean age: 32.8±SD10.9; BMI: 22.4±SD2.7) from Shanghai, China was presented.

The main outcome measures: total acceptability guidelines of the Mediterranean diet (MD acceptability), a 14-item PREDIMED score (MDPS) and perceived barriers to healthy eating (PBHE) were used to compare the two ethnic groups. Within the same ethnicity, the group that consumed olive oil and walnuts was also compared with the group that do not consume these foods by assessing and comparing PBHE, MDPS and MD acceptability. In addition, the association between PBHE and variables including age, BMI, eat out frequency, MD acceptability, MDPS, consumption of olive oil and walnuts and ethnicity was analysed.

Conclusion.

This Bristol Online Survey of Caucasians (n=142) and East Asians (n=142) adults with similar age, BMI suggested that overall East Asians have a better health indicator than

Caucasians as East Asians is associated with a greater MD score and a higher MD acceptability.

Walnuts consumption was higher among older age Caucasians and in lower BMI East Asians population. Olive oil consumption in both ethnicities, especially in Caucasians is closely positively associated with older age, higher MD score, higher MD acceptability and lower PBHE among all three interventions. East Asians reported as eating out more than Caucasians due to cultural behaviour.

Olive oil consumption should be highly recommended to consume among Asians as well as Caucasians. Future clinical trials should focus on identifying what positive health effects will be achieved after olive oil consumption between Asians and Caucasians.

2.1 Introduction

2.1.1 Mediterranean diet acceptability

A Mediterranean style diet is likely to produce a beneficial effect on the occurrence of several chronic diseases, primarily CVD, which are closely linked to lifestyle and eating habits (Rees, Hartley et al. 2012). Systematic reviews of observational prospective studies have reported that greater adherence to a Mediterranean diet (MD) is associated with a significant improvement in health status and a significant reduction in overall mortality, as well as in morbidity and mortality from CVD and other chronic diseases (Sofi, Cesari et al. 2008, Sofi, Abbate et al. 2010). A 2-point increase (scale from 0 to 7-9 points) in adherence to a Mediterranean dietary pattern was associated with an 8% reduction in all-cause mortality, and a 10% reduction in CVD incidence or mortality (Sofi, Abbate et al. 2010).

The feasibility to implement a Mediterranean diet with high olive oil and nuts consumption beyond the Mediterranean regions such as China or Western countries has remained controversial and worthy of follow-up (Murphy and Parletta 2018). One previous questionnaire from Glasgow (Papadaki and Scott 2002) reported that due to the barriers of high price, limited availability and poor quality of familiar foods in the UK, it is difficult to maintain healthy eating behaviours when Greek people migrated to the UK. Similarly, a relatively current qualitative research (Papadaki, Thanasoulis et al. 2016) in Southwest England found that MD was challenging for adults as cost, taste and cooking skills can be considered as adherence barriers in different workplaces. In addition, barriers concerning MD adoption were also identified as perceived difficulty living in a colder climate, perceived impact on body weight, acceptability of a MD and cultural differences among adults with high CVD risk from Northern Europe (Moore 2017). However, a previous study (Papadaki and Scott 2008) from Scotland found that the 6-month MD consumption was successful at sustaining and MD improved blood lipids profile after 3-month follow-up. Additionally, patients with diagnosis of CHD from a Northern European population were reported to have a significant increase ($P < 0.01$) in Mediterranean diet score (MDS) at 6 and 12 months follow-up (Logan, Woodside et al. 2010).

The reason why the Mediterranean diet is difficult to be widely adopted is because the Mediterranean diet is not simply a list of foods. It encompasses a social, cultural, and agricultural way of life (Murphy and Parletta 2018). In addition, Western countries like the UK are among the highest global consumers of meat. Therefore, it was not surprising that one of the dietary instructions difficult for participants to adhere to was restricting red meat

intake (Davis, Hodgson et al. 2017). Nevertheless, it is currently believed that MD has a relatively high compliance outside the Mediterranean region (Murphy and Parletta 2018).

Comparing with the Mediterranean diet, which favours simple meals with earthy, light flavours, Asian diets tend to focus on a balance between salty, sweet, sour and spicy flavours, as well as crunchy and soft textures. Asians tend to favour strong flavours and use rich sauces and infused oils to dress their foods. However, the Chinese diet was shown to shift away from the traditional Chinese diet toward high-fat, low-carbohydrate and low-fibre diets (Zhang, Wang et al. 2015). Limitation of trans fat to below 1% of daily calories is recommended (European Commission 2011), however, Asian diets incorporate unhealthy oils into cooking, especially in restaurant, such as those containing trans-fat such as margarine, maize oil, ground hook oil and animal fat such as lard. Oil-bearing processed food including cookies, cakes, bread, fried chips, popcorn, instant noodles, non-dairy creamer, whipped topping and ice-cream which contains trans fatty acid (TFA) are likely to be the most essential source of hydrogenated vegetable oil consumption for Chinese (Jiang, Xia et al. 2013). Research (Dhaka, Gulia et al. 2011) indicated the direct association of trans fatty acids with cardiovascular diseases, disorders of nervous system, diabetes and obesity. Natural fats and oils are a combination of monounsaturated, polyunsaturated and saturated fatty acids while trans fatty acids are unsaturated fatty acids that contain at least one double bond in the trans configuration. Trans fats are also increasingly part of packaged foods in Asia due to their ability to prolong shelf life, yet there are no laws requiring trans-fat content be disclosed to the public (T.H.Chan 2018). Nevertheless, food-based dietary guidance from China only suggested no more than 25–30 g of cooking oil (Wang, Lay et al. 2016) but still lack suggestion to consume healthy oil such as olive oil.

Although East Asian and Western diets remain significantly different in macro- and micro-nutrient composition, both dietary patterns have aspects that can be considered, respectively, as adverse and protective in relation to the major adult cardiovascular diseases. In both Asian and Western countries, as dietary-associated risk is the most important behavioural factor influencing global health, it appears the best target in the challenge against CVD (Zhou, Stamler et al. 2003).

Table 2.1 compares three major dietary recommendations in Mediterranean areas, UK and China. **Table 2.1** showed that Mediterranean diet with more vegetables, olive oil, nuts consumption exerts healthier dietary pattern than other two diets.

Table 2.1. Comparison of current dietary intakes recommendations of Chinese populations versus UK populations versus Mediterranean diet pyramids

Foods	Mediterranean Diet (PREDIMED study)	British Diet	Chinese Diet
Fruits	≥3 units per day (including natural fruit juices)	≥ 5 portions of fruits and vegetables (each portion 80g) per day	200 – 350 g per day
Vegetables	≥2 servings (≥1 portion raw or as a salad) (1 serving : 200 g)		300 - 500 g per day
Legumes (e.g., kidney beans, lentils or chickpeas)	≥3 servings per week (1 serving:150 g)	N/A	250 – 400 g per day
Nuts, walnuts, pulses	3-4 servings a week	≥ 2 portions a week	Soybean and nuts: 50 – 150 g per day
Oil	Olive oil as the main added lipid: ≥4 tbsp daily	Vegetable oil, rapeseed oil and sunflower oil sparingly	Cooking oil (pork oil: 25-30g) per day
Fish or shellfish	≥3 servings per week (1 serving 140g of fish or 4–5 units or 200 g of shellfish)	≥ 2 portions (each portion 140 g) a week	40 -75 g per day
Poultry	1-3 times a week	≥ 2 portions a week	40 -75 g per day
Dairy	2 servings a day	Lower fat options when possible	300 g per day
Red meat, hamburger, or meat products	<1 serving per day (1 serving: 100–150 g)	No more than 70g per day	Lean meat: 40-75 g per day
Sweets or carbonated beverages	<1 serving per day	Consume infrequently and in a small amount	N/A
Butter, margarine, or cream	<1 serving per day (1 serving: 12 g) (1 serving = 150ml)	N/A	N/A

Commercial sweets or pastries	<3 times per week	N/A	N/A
Water	1.5 to 2.0 L a day	6-8 glasses a day	1500 – 1700 ml per day
Wine	≥7 glasses per day (1 serving = 125ml)	Less than 175ml of wine or 568ml of larger or ale or 568l of cider or 25ml of spirits	N/A
Physical activities	Regular practice of moderate physical activity (at least 30 minutes throughout the day)	At least 150 minutes of moderate intensity activity –30 minutes at least 5 days per week	6000 walking steps per day

2.1.2 Olive oil acceptability

Olive oil, a main component of Mediterranean diet, depending on its origin and extraction process, is high in monounsaturated fatty acids (MUFAs) such as oleic acid (55-83%) containing low levels of α -linolenic acid (ALA) - a member of the omega-3 family of fats (Smith, Kelly et al. 2003) and α ALA (Pan, Chen et al. 2012). Due to its high concentration of phenol compounds, virgin olive oil tastes bitter and pungent therefore many people do not like to consume it (Vitaglione, Savarese et al. 2015). Extra-Virgin olive oil (EVOO), which is considered an unrefined oil since it's not treated with chemicals or altered by temperature, contains most of the natural vitamins and minerals found in olive oil.

There is evidence indicating growing appeal and potentially acceptability of olive oil outside Mediterranean countries. Olive oil imports to China is reported to be 43,400 metric tons in the year of 2013, and an increase of 5.8% from the previous year, up 13% from the same period a year earlier despite the fact that olive oil accounted for only 1% of China's total edible oil consumption in 2013. In 2017, the National Institute of Italian Statistics showed that Italian olive oil exports to China increased by €40 million (Philip 2018). China, as a non-Mediterranean country with non-Mediterranean climate, is taking olive cultivation as an important part of its agricultural development (Su, Sun et al. 2018). In China, the olive industries have been concentrated in several western provinces in Gansu, Sichuan, Yunnan, Chongqing, and Hubei. Although the olive oil business started to play an important role in the economic and social development of western China in recent years, the business scope of olive enterprises is still narrow, the scale of enterprises is commonly small (Su, Sun et al. 2018).

The culinary use of olive oil is relatively new to UK consumers and is regarded as a set of particular attributes rather than as an everyday cooking oil (Garcia, Aragonés et al. 2002). Currently, olive oil has become an established commodity in the UK. The UK's imports of olive oil in 2019 exceeded 84 thousand tonnes, at a value of €220 million. Between 2015 and 2019, imports increased annually by 5% in volume, but decreased in value by -2%. This decrease in import value reflects the lower import prices, especially during 2019 (CBI - Ministry of Foreign Affairs 2020).

Evidence indicated that citizens in Uruguay, which is an emerging olive-growing country, are unacquainted with the sensory traits of extra virgin olive oil, and prefer defective, ordinary virgin olive oils (Gámbaro, Ellis et al. 2013). It also indicated that habituation to the bitter and pungent VOO is possible (Vitaglione, Savarese et al. 2015). However, bitterness and pungency can be the sensory drivers toward a healthy choice and are expected to be increased in the market as an effective strategy to direct consumers choices (Vitaglione, Savarese et al. 2015). People with a higher concern toward health related issues are more likely to consume olive oil, but subjective knowledge is shown to be an important factor (Cavallo 2015).

2.1.3 Nuts acceptability

Nuts are not only tasty, but also provide numerous health benefits. Nuts contain fatty acids such as omega 3, that help lower cholesterol, prevent heart disease, and control diabetes. There are several nuts that have a high concentration of omega-3 fatty acids, adding a daily handful to your diet can deliver health benefits (Chang, Alasalvar et al. 2016). Walnuts have more omega-3 than any other nut. Just 1/4 cup of walnuts provides 2.5 g of the essential fatty acid. Data presented in **Chapter 3** provide evidence on the beneficial effects of nuts consumption on blood lipids, lipids particles and endothelial function.

In the UK, 44% of existing buyers are buying nuts as an alternative to snacks. China is one of the main exporting almonds country with a growth of 40.063 import from 2004 to 2014 (International Nuts & Dried Fruit 2016). Nuts sales have been soaring as the nuts and seeds market in the UK registered a positive compound annual growth rate of 6.28% during the period 2012 to 2017 with a sales value of GBP 538.73 Million in 2017, an increase of 5.74% over 2016. The market achieved its strongest performance in 2016, when it grew by 6.48% over its previous year and its weakest performance in 2017, when it increased by 5.74% over 2016 (Global Data - Research And Markets 2020). The data reported the average purchase weekly of nuts and edible seeds is 42 g in the UK in 2018 (Nils-Gerrit 2020).

2.1.4 Purpose of this survey chapter

This online exploratory survey study titled “Public Opinions of the Mediterranean Diet” aims to evaluate consumption, feasibility and acceptability of olive oil as well as walnuts among adult individuals in the population with an emphasis on Caucasian and East Asian. The association between foods and indices of health such as age, BMI, MD score, MD acceptability and PBHE was investigated.

This survey, however, further informs the planned human trials which aim to test effectiveness of interventions with either nuts or olive oil, compared with a control group, on CV risk factors outcomes between East Asians and Caucasians.

2.2 Methods

From August 2017 to April 2018, “Public Opinions of the Mediterranean Diet” from the online survey (formerly BOS) was conducted and researched around the UK. This study was approved by the Northumbria University ethics committee (**Registration no. 000892**) (**Appendix A3**). A representative sample of 284 respondents in total (142 respondents each for Caucasians and East Asians) who complete the online survey (formerly BOS) with the similar range of body mass index (BMI) was analysed.

2.2.1 Study design

The study was an observational, cross-sectional online survey which aimed to collect data on dietary habits, perceived barriers to healthy eating (PBHE), health condition, PREDIMED scores and total acceptability of Mediterranean dietary guidelines. Given the type of study design, this web-based survey chapter is reported following the STROBE statement (von Elm, Altman et al. 2007) and the CHERRIES statement (Eysenbach 2004). The checklists for these are included in **Appendix A5 and A6** respectively.

2.2.2 Sample

142 Caucasian responses and 142 Asian responses from UK and Shanghai, China were recruited and analysed in this online survey study. By design, participants of this study had to have regular access to internet and email and, no other inclusion/exclusion criteria were

employed. Recruitment was supported by Northumbria University. The study was also advertised among staffs and students at Northumbria University. A “snowball” sampling procedure was attempted by requesting participants to forward the invitation to join the study to others. Participants of this study were recruited in a wide range of individuals in terms of education, residence, marital and socioeconomic status, age and ethnicity.

The recruitment procedure involved two phases. The survey targeted East Asian individuals in China, Shanghai. Afterwards, a matched sample of UK based Caucasian individuals, was recruited as stated above. These samples were matched for age, body mass index and gender balance (this is, similar proportions of men and women).

2.2.3 E-survey

The survey was developed using Online survey (formerly BOS) for the creation of web forms. Participants volunteering to take part in this survey received the uniform resource locator (URL) for the survey. Pilot testing of the survey materials for clarity, understanding and time taken to complete the survey was undertaken prior to the current survey.

2.2.4 Questionnaire measures

The questionnaire survey (**Appendix A3**) comprised 20 questions regarding BMI, alcohol consumption frequency, smoking status, and current health diagnosis, dietary habits, eating barriers, as well as acceptability of Mediterranean dietary guidelines. In addition, a number of questions on sociodemographic characteristics (gender, age, ethnicity, residence, marital status, employment and highest educational attainment) were presented at the beginning of the survey in order to characterize individuals.

The online survey included the following questionnaires: Firstly, questions from first to 23rd on individual characteristics, demographic information were requested. BMI was estimated from self-reported weight and height. Secondly, MD adherence was assessed using the 14-item PREDIMED score (MDPS) from questions 24th to 27th (Schroder, Fitó et al. 2011). The range of possible scores for MDPS is 0-14 with higher scores indicating greater MD adherence. The baseline 14-item questionnaire (**Appendix A3**) was the primary measure used in this study to appraise adherence of participants to the Mediterranean diet. One additional item investigated “How many servings of walnuts (1 serving = 40g) do you consume per week?” Thirdly, a questionnaire requesting participants to identify PBHE from

previously published list derived from a pan-EU consumer attitudinal survey (Kearney and McElhone 2007).

2.2.5 Statistical analysis

Body mass index (BMI) was calculated as weight in kilograms divided by the squared height in metres. Adherence to the Mediterranean diet was assessed using the 14-item PREDIMED score (MDPS) with a range of possible values from 0 to 14, with higher MDPS indicating higher adherence, the number of perceived barriers to healthy eating was estimated adding up all the barriers indicated by the participant and total acceptability of Med diet was calculated out of acceptability for each of the items comprised in MDPS with potential values ranging from 0 to 14. were derived to compare between Caucasian and East Asian population. Items such as age, BMI, MDPS, PBHE and MD total acceptability of Caucasian and East Asian were compared on the basis of whether participants who consumed or not walnuts and olive oil were used to be compared between Caucasian and East Asian responses who did not consume walnuts and olive oil. Furthermore, the association between perceived barriers to healthy eating and age, BMI, MDPS, eat out frequency, total acceptability of MD guidance, olive oil, walnuts was analysed among Caucasian and East Asian population. Finally, the association between Caucasian/East Asian who consume olive oil, walnuts or not and socio-status also was analysed.

All statistical analyses were conducted with “IBM SPSS Statistics 21” together with Microsoft Office Excel 2013” for Windows. Descriptive statistics with mean and standard deviations were used to summarise the characteristics of the participants in the study. Two-step cluster-analysis was used to identify natural groups of PBHE. Step one involved creation of pre-clusters from cases by constructing an algorithm known as the Cluster feature tree. In Step two, pre-clusters were merged using agglomerative hierarchical clustering. Cluster-membership and the optimal number of clusters were determined using model fit indices including log-likelihood distance measure and the Schwarz’s Bayesian information criterion.

2.3 Results

2.3.1 Participant characteristics

A representative sample of 21 to 67 years old 284 respondents in total (142 respondents each for Caucasians and East Asians) who complete the Online Survey have similar range of body mass index (BMI) (Table 2.2).

Ethnicity		N	Minimum	Maximum	Mean±SD
Caucasian	Age	142	21	67	32.8±12.2
	BMI	142	17.8	33.6	22.8±2.8
	Female	124	87.3%		
East Asian	Age	142	21	68	32.8±10.9
	BMI	142	17.2	33.4	22.4±2.7
	Female	111	78.2%		

Independent-samples T-test

2.3.2. The association between interventions - olive oil and walnuts, and BMI, PREDIMED score, barriers and MD acceptability among ethnicities.

Table 2.3.1 shows that East Asians are overall healthier than Caucasians as MD score ($p < 0.001$), and that East Asians have a higher eat out frequency ($p < 0.001$) and MD acceptability ($p = 0.028$) are statistically significantly different except PBHE.

In Table 2.3.2, Caucasians who consume walnuts ($n = 18$) are more likely to eat out frequently ($p = 0.043$) than Caucasians who do not consume walnuts ($n = 124$). East Asians with older age ($35.51 \pm SD 13.0$) are more likely to consume walnuts ($p = 0.028$).

In Table 2.3.3, Caucasians ($n = 104$) who consume olive oil were significantly older ($34.3 \pm SD 12.4$) ($p = 0.009$), scored higher for MD adherence score ($6.51 \pm SD 2.2$) ($p < 0.001$), scored higher for MD acceptability ($10.21 \pm SD 2.3$) ($p = 0.017$) and reported lower PBHE ($1.81 \pm SD 4.0$) ($p = 0.03$) than non-consumers of olive oil. East Asians olive oil consumers ($n = 44$) is positively associated with higher MD score ($8.02 \pm SD 1.8$) than those non-olive oil consumers ($n = 98$) who have lower MD score ($6.83 \pm SD 1.6$) ($p < 0.001$).

In Table 2.3.4, Non-walnuts East Asians consumers also show a higher MD score ($7.17 \pm SD 2$; $p < 0.001$), higher eat out frequency ($14.29 \pm SD 15.9$; $p < 0.001$) and higher total MD acceptability ($10.85 \pm SD 3.3$; $p = 0.032$) than non-walnuts Caucasians consumers. Non-olive oil East Asians consumers also show a higher eat out frequency ($13.66 \pm SD 13.3$;

P<0.001), higher MDPS (6.83±SD1.7; p<0.001) and higher MD acceptability (10.73±SD3.1; P=0.001) than non-olive oil Caucasians consumers.

Among all walnuts' consumers in both ethnicities, East Asians walnuts consumers also show lower BMI (22.27±SD2.5; p=0.042) and higher eat out frequency (13.82±SD11; p<0.001) (**Table 2.3.4**). Among all olive oil consumers in both ethnicities, East Asians olive oil consumers show a higher eat out frequency (15.09±SD16.1; P<0.001) and higher MD score (8.02±SD1.8; P<0.001) than Caucasians olive oil consumers (**Table 2.3.5**).

Table 2.3.1 Perceived health status of differences in scores for perceived barriers (PBHE), adherence to the Mediterranean diet (MD score), total MD acceptability of the Mediterranean diet and eat out frequency between Caucasians and East Asians.

	Ethnicity	N	Mean (SD)	Sig. (2-tailed)
Number of perceive barrier to health eating (PBHE)	Caucasian	142	2.33 (4.2)	0.623
	East Asian	142	2.09 (4.1)	
MD score	Caucasian	142	6.03 (2.3)	<0.001
	East Asian	142	7.20(1.8)	
Eat out frequency (times per month)	Caucasian	142	4.72(4.3)	<0.001
	East Asian	142	14.11(14.2)	
Total acceptability of MD guidelines	Caucasian	142	9.94(2.2)	0.028
	East Asian	142	10.68(3.3)	

Table 2.3.2 Perceived health status of differences between walnuts consumption and scores for perceived barriers (PBHE), adherence to the Mediterranean diet (MD score), total MD acceptability of the Mediterranean guidelines and eat out frequency for Caucasians and East Asians.

Ethnicity		Walnuts	N	Mean (SD)	P value
Caucasian	Number of perceive barrier to health eating	No	124	2.44(4.3)	0398
		Yes	18	1.56(3.0)	
	MD score	No	124	5.99(2.3)	0.618
		Yes	18	6.28(2.1)	
	Eat out frequency (times per month)	No	124	4.44(4.0)	0.043
		Yes	18	6.61(5.3)	
	BMI	No	124	22.69(2.9)	0.207
		Yes	18	23.57(1.7)	
	Age	No	124	32.45(12.2)	0.399
		Yes	18	35.06(11.8)	
	Total MD acceptability	No	124	9.97(2.2)	0.735
		Yes	18	9.78(2.1)	

East Asian	Number of perceive barrier to health eating	No	87	1.97(4.2)	0.643
		Yes	55	2.29(3.9)	
	MD score	No	87	7.17(1.7)	0.836
		Yes	55	7.24(1.9)	
	Eat out frequency (times per month)	No	87	14.29(16.0)	0.849
		Yes	55	13.82(11.1)	
	BMI	No	87	22.55(2.8)	0.560
		Yes	55	22.27(2.5)	
	Age	No	87	31.05(9.0)	0.028
		Yes	55	35.51(13.0)	
	Total MD acceptability	No	87	10.85(3.3)	0.451
		Yes	55	10.42(3.4)	

Table 2.3.3 Perceived health status of differences between olive oil consumption and scores for perceived barriers (PBHE), adherence to the Mediterranean diet (MD score), total MD acceptability of the Mediterranean guidelines and eat out frequency for Caucasians and East Asians.

Ethnicity		Olive oil	N	Mean (SD)	Sig. (2-tailed)
Caucasian	Age	No	38	28.63(10.7)	0.009
		Yes	104	34.30(12.4)	
	BMI	No	38	22.85(2.8)	.908
		Yes	104	22.79(2.7)	
	Eat out frequency (times per month)	No	38	5.11(5.1)	0.515
		Yes	104	4.58(3.9)	
	MD score	No	38	4.71(1.9)	<0.001
		Yes	104	6.51(2.2)	
	Total MD acceptability	No	38	9.21(2.0)	0.017
		Yes	104	10.21(2.3)	
PBHE	No	38	3.76(4.9)	0.03	
	Yes	104	1.81(4.0)		
East Asian	Age	No	98	32.53(10.9)	0.692
		Yes	44	33.32(11.0)	
	BMI	No	98	22.57(2.7)	0.376
		Yes	44	22.14(2.6)	
	Eat out frequency (times per month)	No	98	13.66(13.3)	0.582
		Yes	44	15.09(16.1)	
	MD score	No	98	6.83 (1.6)	<0.001
		Yes	44	8.02 (1.8)	
	Total MD acceptability	No	98	10.73(3.1)	0.783
		Yes	44	10.57(3.7)	

	PBHE	No	98	2.10(4.0)	0.964
		Yes	44	2.07(4.3)	

Table 2.3.4. The association of all MD variables including scores for perceived barriers (PBHE), adherence to the Mediterranean diet (MD score), total MD acceptability of the Mediterranean guidelines and eat out frequency between East Asians and Caucasians who adopt walnuts consumption, and the association of all MD variables between East Asians and Caucasians who do not consume walnuts.

Walnuts consumption		Ethnicity	N	Mean (SD)	Sig. (2-tailed)	
No	Age	Caucasian	124	32.45(12.3)	0.338	
		East Asian	87	31.05(9.0)		
	BMI	Caucasian	124	22.69(2.9)	0.710	
		East Asian	87	22.55(2.8)		
	Eat out frequency (times per month)	Caucasian	124	4.44(4.0)	<0.001	
		East Asian	87	14.29(15.9)		
	MDPS	Caucasian	124	5.99(2.3)	<0.001	
		East Asian	87	7.17(1.7)		
	PBHE	Caucasian	124	2.44(4.3)	0.421	
		East Asian	87	1.97(4.2)		
	MD acceptability	Caucasian	124	9.97(2.2)	0.032	
		East Asian	87	10.85(3.3)		
	Yes	Age	Caucasian	18	35.06(11.8)	0.896
			East Asian	55	35.51(13.0)	
BMI		Caucasian	18	23.57(1.67)	0.042	
		East Asian	55	22.27(2.5)		
Eat out frequency (times per month)		Caucasian	18	6.61(5.3)	<0.001	
		East Asian	55	13.82(11.0)		
MDPS		Caucasian	18	6.28(2.1)	0.072	
		East Asian	55	7.24(1.8)		
PBHE		Caucasian	18	1.56(3.0)	0.469	
		East Asian	55	2.29(4.0)		
MD acceptability		Caucasian	18	9.78(2.1)	0.45	
		East Asian	55	10.42(3.4)		

Independent Sample T-test

Table 2.3.5 The association of all MD variables including scores for perceived barriers (PBHE), adherence to the Mediterranean diet (MD score), total MD acceptability of the Mediterranean guidelines and eat out frequency between East Asians and Caucasians who adopt olive oil consumption, and the association of all MD variables between East Asians and Caucasians who do not consume olive oil.

Olive oil consumption		Ethnicity	N	Mean (SD)	Sig. (2-tailed)	
No	Age	Caucasian	38	28.63(10.7)	0.062	
		East Asian	98	32.53(10.9)		
	BMI	Caucasian	38	22.85(2.8)	0.604	
		East Asian	98	22.57(2.7)		
	Eat out frequency	Caucasian	38	5.11(5.1)	<0.001	
		East Asian	98	13.66(13.3)		
	MDPS	Caucasian	38	4.71(1.9)	<0.001	
		East Asian	98	6.83(1.7)		
	PBHE	Caucasian	38	3.76(5.0)	0.067	
		East Asian	98	2.10(4.0)		
	Total MD acceptability	Caucasian	38	9.21(2.0)	0.001	
		East Asian	98	10.73(3.1)		
	Yes	Age	Caucasian	104	34.30(12.4)	0.635
			East Asian	44	33.32(11.0)	
BMI		Caucasian	104	22.79(2.7)	0.183	
		East Asian	44	22.14(2.6)		
Eat out frequency		Caucasian	104	4.58(4.0)	<0.001	
		East Asian	44	15.09(16.1)		
MDPS		Caucasian	104	6.51(2.2)	<0.001	
		East Asian	44	8.02(1.8)		
PBHE		Caucasian	104	1.81(3.7)	0.712	
		East Asian	44	2.07(4.3)		
Total MD acceptability		Caucasian	104	10.21(2.3)	0.559	
		East Asian	44	10.57(3.7)		

Independent Sample T-test

2.3.3. Mediterranean diet adherence

The mean 14-item Mediterranean diet score (MDPS) was 6.03±SD2.3 for Caucasian and 7.2±SD1.8 for East Asian. East Asians reported the greatest adherence to the following components of the MDPS: “<7/week servings of red meat, hamburger, or meat products” (90.1%), “<7/day servings of sweet or carbonated beverages” (88%) while Caucasians reported the greatest adherence to the following components of the MDPS: “≥2 servings of vegetables a day” (84.5%), “Preferentially consume chicken turkey or rabbit meat instead

of veal, hamburger or sausage” (81.7%), East Asians also reported a higher olive oil consumption: “≥4 tablespoons of olive oil a day (14.8%)” than Caucasians “ ≥4 tablespoons of olive oil a day (3.5%)” (**Table 2.4**). There is significant difference of “Used olive oil as main fat for cooking in diet (Yes)” (P < 0.001); “≥4 tablespoons of olive oil a day” between Caucasians and East Asians (P = 0.001); “<7/week servings of red meat, hamburger, or meat products” (P < 0.001); “<1/day servings of butter, margarine, or cream” (P < 0.001); “<7/day servings of sweet or carbonated beverages” (P < 0.001); “≥7/week glasses of wine (P < 0.001)”; “≥3/week servings of fish or shellfish” (P < 0.001); “Preferentially consume chicken turkey or rabbit meat instead of veal, hamburger or sausage (Yes)” (P=0.013) between Caucasians and East Asians (**Table 2.4**).

Table 2.4. Adherence to the individual components of the Mediterranean diet assessed using the PREDIMED score (MDPS).			
	Caucasian	East Asian	
	(n = 142)	(n = 142)	
	LSM±SD	LSM±SD	
PREDIMED score (MDPS)	6.03±2.3	7.2±1.8	
	%	%	P-value
(1) Used olive oil as main fat for cooking in diet (Yes)	73.2	31.0	< 0.001
(2) ≥4 tablespoons of olive oil a day	3.5	14.8	0.001
(3) ≥2 servings of vegetables a day (1 serving = 80g or 2 broccoil florets, three heaped tablespoons of cooked vegetables such as carrots or 3 celery sticks)	84.5	81.0	0.432
(4) ≥3 servings of fruit a day (1 serving =80g, or 1 banana, 2 kiwis, or 1 heaped tablespoon of sultanas)	30.3	26.8	0.511
(5) <7/week servings of red meat, hamburger, or meat products (1 serving =70g, or three slices of ham)	31.7	90.1	< 0.001
(6) <1/day servings of butter, margarine, or cream (1 serving =10g)	35.9	65.5	< 0.001
(7) <7/day servings of sweet or carbonated beverages (1 serving =150ml)	41.5	88.0	< 0.001
(8) ≥7/week glasses of wine (1 serving = 125ml)	9.9	0	< 0.001

(9) ≥3/week servings of legumes/pulses (1 serving =5 heaped tablespoons cooked)	29.6	25.4	0.425
(10) ≥3/week servings of fish or shellfish (1 serving =140g)	23.2	57.7	< 0.001
(11) <3/week servings of cakes, commercial sweets or pastries	46.5	56.3	0.096
(12) ≥3/week servings of unsalted nuts including peanuts (1 serving =40g)	21.8	30.3	0.105
(13) Preferentially consume chicken turkey or rabbit meat instead of veal, hamburger or sausage (Yes)	81.7	69	0.013
(14) ≥3/week consume a meal containing vegetables, pasta, rice, or other dishes seasoned with tomato based sauce	21.8	30.3	0.105

2.3.4. PBHE classification and cluster characteristics

Two-step cluster analysis produced three discrete clusters of participants based on their reports of number of perceived barriers to healthy eating (PBHE) (**Table 2.5.1; Table 2.5.2**). Cluster-1 (n=102) having the largest number of participants was characterised by PBHE as “No barriers”. The important PBHE for participants in cluster-3 (n = 21) included “Price”, “Perishable” and “Dining out”. Participants in cluster-2 (n=19) reported the importance barriers on “Busy lifestyle”, “Working hours” and “Willpower”.

On average, members of cluster-3 were youngest (mean = 30.9 years for Caucasians, mean = 29.2 years for East Asians) and reported the lowest MDPS (mean = 4.25), highest PBHE (mean = 11 in Caucasians; mean = 10.6 in East Asians) as well as lowest total MD acceptability (mean=9.6 in Caucasians; mean=9.1 in East Asians) and the most frequency of eating out (mean = 26.4) in East Asians (**Table 2.5**). In **Table 2.5**, On the contrary, members of cluster-1 with “No barriers” were the leanest (mean BMI = 22.4 kg/m² for Caucasians: mean BMI = 22.3 kg/m² for East Asians) group, reporting a lowest number of PBHE (Mean < 0.01), highest score in MDPS (mean = 7.5) and the least frequency of eating out (mean = 4.3).

Table 2.5 Characteristics of PBHE clusters.

Ethnicity			N	Mean	Std. Deviation	Sig
Caucasian	Total MD acceptability	Cluster 1	102	10.04	2.3	0.697
		Cluster 2	19	9.7	1.5	
		Cluster 3	21	9.6	2.2	
		Total	142	9.9	2.2	
	PBHE	Cluster 1	102	0.01	0.1	<0.001
		Cluster 2	19	5.2	1.8	
		Cluster 3	21	11.0	2.0	
		Total	142	2.3	4.1	
	MDPS	Cluster 1	102	6.6	2.2	<0.001
		Cluster 2	19	5.00	1.9	
		Cluster 3	21	4.05	1.5	
		Total	142	6.03	2.3	
	BMI	Cluster 1	102	22.4	2.2	0.025
		Cluster 2	19	23.3	3.3	
		Cluster 3	21	24.1	4.1	
		Total	142	22.8	2.7	
	Age	Cluster 1	102	33.1	12.1	0.731
		Cluster 2	19	33.5	15.8	
		Cluster 3	21	30.9	9.2	
		Total	142	32.8	12.2	
Eat out frequency	Cluster 1	102	4.3	3.7	0.035	
	Cluster 2	19	7.0	7.0		
	Cluster 3	21	4.9	2.9		
	Total	142	4.7	4.3		
East Asian	Total MD acceptability	Cluster 1	105	10.9	3.4	0.054
		Cluster 2	17	11.2	2.8	
		Cluster 3	20	9.1	2.9	
		Total	142	10.7	3.3	
	PBHE	Cluster 1	105	0.00	0.0	<0.001
		Cluster 2	17	5.0	1.9	
		Cluster 3	20	10.6	3.3	
		Total	142	2.1	4.1	
	MDPS	Cluster 1	105	7.5	1.6	<0.001
		Cluster 2	17	6.7	2.3	
		Cluster 3	20	5.8	1.6	
		Total	142	7.2	1.8	
	BMI	Cluster 1	105	22.3	2.5	0.286
		Cluster 2	17	23.4	3.8	
		Cluster 3	20	22.6	2.8	
		Total	142	22.4	2.7	

	Age	Cluster 1	105	33.1	11.4	0.219
		Cluster 2	17	35.2	10.4	
		Cluster 3	20	29.2	7.4	
		Total	142	32.8	10.9	
	Eat out frequency	Cluster 1	105	11.9	9.9	<0.001
		Cluster 2	17	13.5	8.9	
		Cluster 3	20	26.4	26.6	
		Total	142	14.1	14.2	

Table 2.5.1 Walnuts consumption of Two-Step Cluster between two ethnicities

Ethnicity			Two Step Cluster Number 3			Total	P
			1	2	3		
Caucasian	Walnuts	No	88	16	20	124	0.483
		Yes	14	3	1		
	Total	102	19	21	142		
East Asian	Walnuts	No	67	9	11	87	0.573
		Yes	38	8	9		
	Total	105	17	20	142		

Two Step Cluster Chi-Square Tests

Table 2.5.2 Olive oil consumption of Two-Step Cluster between two ethnicities

Ethnicity			Two Step Cluster Number 3			Total	P
			1	2	3		
Caucasian	Olive oil	No	22	8	8	38	0.080
		Yes	80	11	13		
	Total	102	19	21	142		
East Asian	Olive oil	No	72	13	13	98	0.740
		Yes	33	4	7		
	Total	105	17	20	142		

Two Step Cluster Chi-Square Tests

2.4 Discussion

2.4.1 Principal findings

High levels of acceptability of the Mediterranean dietary guidelines were observed among both ethnic groups. East Asians participants had slightly but significantly greater adherence to the Mediterranean dietary guidelines, as well as acceptability of these guidelines than Caucasians participants in this survey study.

The acceptability and frequency of olive oil intake among East Asians is higher than Caucasians. Caucasians who consume olive oil were significantly older, scored higher for MD adherence score, scored higher for MD acceptability and reported lower PBHE than non-consumers of olive oil. Walnuts are most consumed by older age among Caucasians as well as lower BMI East Asians population. Olive oil consumption has overall beneficial effect on both ethnicities (mostly female: 87.3% in Caucasians, 78.2% in East Asians), and olive oil consumption is most closely positively associated with older age, higher MD score, higher MD acceptability and lower PBHE in both ethnicities. In addition, in this survey, East Asians are shown to be more likely to eat out more frequently than Caucasians because as of cultural phenomenon that Chinese habitually treat others with meals in order to establish and maintain, enhance interpersonal relationship. Owing to its function to express the central position in the representation and relationship, a dinner or banquet can be used as a symbol of the important events in human life, such as wedding, baptism, and religious belief in China (Ma 2015). Taken together these results suggest that interventions promoting the Mediterranean dietary guidelines are likely to be acceptable among these individuals.

MD scores (score ≥ 7 based on the score ranging from 0-14) are associated with older age, waist circumference, body mass index, high education level (Acar, Gucuk Ipek et al. 2018). Previous evidence (Sofi, Cesari et al. 2008) reported that greater adherence to a Mediterranean diet is closely associated with a significant improvement in health status, as seen by a significant reduction in overall mortality (9%), mortality from cardiovascular diseases (9%), and incidence of Parkinson's disease and Alzheimer's disease (13%) (Sofi, Cesari et al. 2008). These consequences appear to be clinically relevant for public health, in particular for encouraging a Mediterranean-like dietary pattern for primary prevention of major chronic diseases (Sofi, Cesari et al. 2008, Sofi, Abbate et al. 2009).

Given the strong evidence supporting its efficacy and effectiveness in enhancing health, the MD is the dietary pattern of choice when promoting a healthy diet (Martínez-González, García-Arellano et al. 2012, Estruch, Ros et al. 2013). The PREDIMED study displayed that closer adherence to a MD, e.g. a MDPS of 9 or higher, was associated with significantly lower odds ratios for prevalence of obesity and risk of cardiovascular diseases (Martínez-González, García-Arellano et al. 2012, Estruch, Ros et al. 2013).

Although the East Asians scored higher than the Caucasians, both groups are below the scores cut offs suggested as both ethnicities would need to improve in order to get protections from CVD risk. This is not unexpected among individuals from non-

Mediterranean countries who are unlikely to score high on the MD score. Consequently, this result justifies the need for dietary interventions to improve the diet of these non-Mediterranean area groups in order to promote health and prevent chronic diseases such as cardiovascular diseases.

The data from this survey research suggested however that East Asian participants (MDPS = 7.2 ± 1.8 for East Asians) are less likely to suffer from cardiovascular diseases than Caucasians (MDPS = 6.03 ± 2.3 for Caucasians). Importantly, evidence shows that small changes such as a two-point increase in MDPS is strongly associated with lower health risks (Salas-Salvadó, Bulló et al. 2011, Estruch, Ros et al. 2013).

Based on the cluster analysis, barriers were identified that could potentially limit the adoption of healthy eating habits. The pan-European consumer attitudinal survey identified “time” as well as “taste” as the most frequent PBHE among the EU population (Kearney and McElhone 2007). A telephone-survey in Northern Ireland reported that ‘lack of willpower’ and ‘willingness to change’, were the most common PBHE (Appleton, McGill et al. 2010). A study offering a “healthy diet” for a 3-day duration to a small group of Scottish middle-age adults, identified “time pressures”, “desire for convenience” and “lack of motivation to cook” as important PBHE (Macdiarmid, Loe et al. 2013). It is also reported that “busy lifestyle”, “irregular working hours”, and “the belief that healthy eating involves lengthy preparation”, are key barriers as identified by the younger, more overweight individuals with the lowest MDPS and HBS. These “time pressure related” PBHE were reported to be likely to influence health behaviours such as reducing physical activity and contribute to the greater prevalence of health risk factors including increasing BMI among participants in that cluster (Lara, McCrum et al. 2014). The same study also showed that cluster-2 identified “lack of willpower” as did our study and “finding it hard to give-up liked foods” as a characteristic PBHE identified by older, but leaner participants with higher MDPS and fewer PBHE (Lara, McCrum et al. 2014). A meta-analysis (Carpenter 2010) has shown perceived barriers and benefits were consistently the strongest predictor of whether an individual adopted a preventative health measure.

Further studies (Seguin, Connor et al. 2014) have reported that the largest barrier is concerning price and lacking in healthy eating knowledge. Firstly, this barrier may raise the question as to whether some respondents might claim these as barriers because they are aware that they probably have a health issue needing personal investments (time, money, convenience), but doubt their own ability or true intention to act on it especially when they

are young. Secondly, the economic barrier was regarded as a culprit to healthy eating. Evidence indicated that price modifications are more effective than educational health information to motivate people to purchase healthier foods, especially for young people. Food prices have increased, with costs 8% higher in real terms than they were in 2007 (Department for Environment 2015). Since 2008, the price of food has risen 10% more than other goods (Griffith, O'Connell et al. 2015). Excluding food bought out of the home, the average household spends 11% of their income on food. This is 16% for low-income households, who now spend 23% more on food than they did in 2007, compared to the average increase of 18%. Thirdly, the barrier “perishable” is likely due to lack of cooking skills and food knowledge among young people. Although nationally representative survey data found that 89% of respondents said they were able to cook a main dish from basic ingredients without help (Adams, Goffe et al. 2015), higher ultra-processed foods consumption is associated with erosion of home food preparation skills and infrequent use of these skills (Lam and Adams 2017). Then, “Dining out” as a barrier to healthy eating is due to the availability of overwhelming unhealthy food available outside of the home. Fast food consumption is associated with increased BMI, the likelihood of obesity and body fat ratios. The number of food outlets in the UK has increased from 60,760 to 93,285 over the last ten years with more fast-food outlets in deprived areas (UK Parliament 2016). Take away foods may also come in larger portions. Additionally, “Will power” is a noticeable barrier to healthy eating and avoid consuming takeaway foods and it reported that people regard healthy eating as difficult to achieve and that it requires great psychological effort to maintain a sustainable healthy diet (Vélez-Toral, Rodríguez-Reinado et al. 2020).

Asians were shown to have a greater interest in eating healthy than westerners according to a survey report which included 1300 responders from Asia and the Western hemisphere (Bary 2017). Around seven in ten of Asian respondents surveyed (68%) were “very interested” in healthy eating, as opposed to 38% of western citizens. In addition, 39% Asian consumers acknowledged that cutting down on their consumption of meat was crucial to achieving a healthy diet and Asian purchasers appeared almost three times likelier (28%) than Westerners (10%) to be more willing to purchase a product if it made vegetarian or vegan health claims. On the contrary, only 25% of Western consumers agreed with this stance (Bary 2017).

Furthermore, another study (Leung and Stanner 2011) reported that Chinese are more likely to be not influenced or restricted by religion on food choices than Westerners and have wide acceptability of testing and tasting wide range of food types. Chinese adults were less likely

to report limiting long-standing and psychological illnesses compared with the general population (Heim and MacAskill 2006).

Dining out frequently is strongly associated with unhealthy lifestyle and uncontrolled dietary intake. Individuals who go out to eat end up taking in typically larger than 3.3 ounces per meal and taking in an average of 200 more calories than those that away-from-home-dining in restaurants, in fast food outlets, and from take-out meals increases the risk of cardiovascular diseases and stroke (Haring et al, 2015).

Study reported that the hyperlipidemic subjects, both men and women, tended to dine out often and consume more animal-based foods and alcohol (Deng et al, 2012). Systematic review found that eating out of the home was strongly associated with an overall higher total calorie and fat intake, and greater portion sizes than ones served at home because people are more likely to choose “unhealthy” foods as eating out is seen as a “treat” with friends (Lachat et al, 2011). Additionally, there is no control over cooking methods or added ingredients, such as fat or salt. Research also showed that dining out makes more of a chance of eating extras such as entrees and desserts, if alcohol is taken with a meal, this may encourage us to eat more. Therefore, reduce the number of times a month for dining out is considered to be healthy for people’s lifestyle (Lachat et al, 2011).

2.4.2 Strengths and limitations

A strength of this relies on the fact that it involved the same number of groups of Asian (n=142) and Caucasian individuals (n=142) matched for age and BMI and gender balance (participation), in order to minimise the confounding impact from these variables. As expected, this online survey produced a good response rate and collection of data at virtually no cost. Another strength of the present survey study is that data were collected via the online surveys, which is a validated, powerful data collection tool designed for experienced organization Academic Research, Education and Public Sector organisations. Among the limitations of this study are the fact that the cross-sectional study design does not allow to establish cause-and effect. In addition, self-reported information is susceptible to recall and social biases such as social desirability (Lara, Scott et al. 2004). A general tendency to underreport and misreport weight, and to overreport height, servings of the foodstuffs could introduce bias to the self-reported lifestyle risk factors (Jarbøl, Larsen et al. 2017). The differences between Asians and Caucasians could be related to the possibility of including more Asians individuals who were health motivated. As it is common with diet-related research, the majority of participants were women (87.3% in Caucasians, 78.2% in East Asians), this making difficult the generalisation of our findings to the male population.

Gender difference exists in perceived healthiness of food within Chinese and Caucasians society (Ma 2015, Bärebring et al., 2020). Chinese male members within the family are given more food with excessive calories as compared with the female members. Such long-term sex difference in food distribution in most Chinese families causes different dietary behaviour between two genders (Ma 2015). Swedish female residents were more likely than men to avoid eating gluten, red meat, white flour and food additives due to perceived unhealthiness (Bärebring et al., 2020). Therefore, this results further informs the design of our human clinical trial to test the effects of either olive oil or nuts in male participants.

2.5. Conclusions

In conclusion, this survey study was well received and ascertained to be an efficient approach to screen dietary habits, PBHE, and perceptions of health status between Caucasians and East Asians. The results of our study are in agreement with our hypothesis indicating that individuals reporting more PBHE are likely to have lower MDPS and higher BMI in both ethnicities.

Olive oil consumption in both ethnicities, especially in Caucasians is most closely positively associated with older age, higher MD score, higher MD acceptability and lower PBHE although all three interventions were presented to exert beneficial effects on both ethnicities. This survey study also showed that the acceptability and frequency of olive oil intake among East Asians is higher than Caucasians. These findings may help in developing dietary interventions promoting the MD acceptability, particularly increasing the consumption of olive oil among individuals of both Caucasians and East Asians to promote health benefits.

Research questions raised from the present Chapter 2:

The work described in this chapter raised the following questions:

1. What is the acceptability and frequency consumption of nuts and olive oil consumption among Caucasians, and East Asians from the evidence of the systematic review and meta-analysis?
2. What is the state of the evidence from interventional studies assessing the impact of dietary interventions including nuts and especially olive oil consumption on CVD risk factors?
3. Is the effect of olive oil on cardiovascular risk factors similar between different ethnic populations living in the Northeast England?

CHAPTER 3

Efficacy of nutritional interventions supplementing nuts on cardiovascular risk factors among adults' individuals with two ethnicities – Asian and Non-Asian population: A systematic review and meta-analysis of intervention studies.

Abstract

Background and aims. Consumption of nuts has been associated with a decreased risk of cardiovascular disease events and death. Nevertheless, factors such as ethnicity plays a role in this association is still controversial in clinical trials. Therefore, a systematic review and meta-analysis of the evidence on the effect of supplementing nuts on CV risk factors in different ethnic groups was proposed.

Methods. Medline, Web of Science and Scopus databases were searched from inception to August 2017 to identify interventional trials assessing the impact of different types of nuts on various cardiovascular risk factors. Inclusion criteria were intervention randomized controlled trials reporting effects of nuts on CV risk factors among adults and adolescents. The outcomes of interest included blood lipids (total-, HDL-, LDL-C, triglycerides, VLDL-C), oxidative stress biomarkers (oxidized-LDL), endothelial function (FMD), blood pressure (SBP, DBP) and inflammatory biomarkers (CRP, *hs*-CRP, IL-6, sICAM-1, sVCAM-1, Apo B, Apo A-I and adiponectin). The pooled estimates of mean differences is calculated and their 95% confidence intervals (CIs) by using random-effects models. The protocol has been registered with PROSPERO and the systematic review registration number is **CRD42018089055**.

Results. From a total of 383 publications identified, 82 studies were included in the final selection and were meta-analyzed. Overall, intervention with daily nuts consumption ranging approximately between 4.05g and 128g resulted in a significantly more pronounced decrease in total cholesterol (mean difference: -7.54; 95% CI: -10.2 to -4.89; $p < 0.00001$; $I^2 = 59\%$; $n = 66$ trials), in HDL-cholesterol (mean difference: 0.89; 95% CI: 0.04 to 1.75; $P=0.04$; $I^2 = 53\%$; $n=67$ trials), in LDL- cholesterol (mean difference: -7.21; 95% CI: -9.38 to -5.04; $P < 0.00001$; $I^2 = 68\%$; $n=68$ trials), in TG (mean difference: -8.83; 95% CI: -13.12 to -4.53; $P < 0.0001$; $I^2 = 64\%$; $n=65$ trials), in VLDL-cholesterol (mean difference: -2.25; 95% CI: -3.74 to -0.77; $P=0.003$; $I^2=0\%$; $n=10$ trials), in Apo B (mean difference: -4.47; 95% CI: -7.01 to -1.94; $P=0.0005$; $I^2=64\%$, $n=23$ trials), in FMD (mean difference: 0.74; 95% CI: 0.09

to 1.39; $P=0.03$; $I^2=5\%$, $n=10$ trials) and in FFA (mean difference: -0.03 ; 95% CI: -0.05 to -0.01 ; $P=0.0009$; $I^2=0\%$, $n=4$ trials) as compared to controls, respectively. Subgroup analysis indicated that the improvement on biomarkers of oxidized-LDL, SBP, Apo B, Apo A-I, CRP and sVCAM-1 were more likely to be shown in the Asian population compared with the non-Asian population although such differences were not statistically significant. Non-Asian population is observed to tend to present a better result on TC, HDL-C, LDL-C, TG, VLDL-C, FFA, adiponectin, *hs*-CRP, IL-6 and sICAM-1 after nuts intake than Asian population although there are also no statistically significant differences between two ethnicities.

Conclusions. These results provide evidence on the beneficial effects of nuts on blood lipids, lipids particles and endothelial function. This review also observed no significant different effects after nuts consumption between different ethnicities but showed a valuable tendency of improvement on cardiovascular markers among two ethnicities. The non-Asian group potentially tends to benefit more cardiovascular biomarkers with moderate nut consumption than Asian group. Asian population is likely to show improvements on some inflammatory markers. These results provide valuable knowledge for designing future human clinical trials. However, future large-scale studies need validate and replicate such studies and more studies testing biomarkers need to be required on Asian population with nut consumption in the near future. Studies might also specifically need to focus on identifying the different efficacy of specific type of nuts on different ethnicities.

3.1 Introduction

Globally, total consumption of tree nuts has grown by 59% over the last one decade (International Nuts & Dried Fruit 2016). Pecans (70%), cashews (60%), and macadamia (59 %), followed by almonds experienced the most significant growth in the last 10 years (International Nuts & Dried Fruit 2016). In the UK, according to the latest food report from global research organization, 44% of existing buyers are buying nuts as an alternative to snacks. China is one of the main exporting almonds country in this world and a growth of 40.063 import from 2004 to 2014 was reported from International Nuts council (INC) (International Nuts & Dried Fruit 2016). Therefore, while people consume more nuts, the health benefits of nuts consumption especially for cardiovascular health need to be researched and recognized.

As a type of nutrient-dense food, nuts contain a high total fat and nearly one half of the fatty acid composition of nuts is made up of unsaturated fat, which comprises monounsaturated fatty acids (MUFAs; oleic acid) and mostly polyunsaturated fatty acids (PUFAs) including linoleic acid and α -linolenic acid (ALA), the plant omega-3 fatty acid especially in walnuts (Ros and Mataix 2006, Ros 2009). In addition, nuts are rich sources of bioactive macronutrients too as nuts are an excellent source of protein and often contain a high content of L-arginine, the amino acid precursor of the endogenous vasodilator nitric oxide. Furthermore, nuts are also a good source of dietary fiber ranging from 4 to 11 g/100 g. There are also significant micronutrients among nut constituents. Nuts have sizeable amounts of folate and they are rich sources of antioxidant vitamins and phenolic compounds (Ros 2009).

3.1.1 Nuts and health

Significant evidence from prospective observational studies and clinical trials consistently observed that most nut constituents were shown beneficial effects while clinically tested, in isolation or as part of enriched foods, for effects on diverse cardiovascular outcomes, including both traditional and novel risk markers (Bao, Han et al. 2013, Estruch, Ros et al. 2013, Aune, Keum et al. 2016). Previous systematic reviews of high walnut intake (Banel and Hu 2009) and of moderate tree nuts intake (Del Gobbo, Falk et al. 2015) significantly

resulted in a decrease of total and LDL cholesterol for 4-24 weeks of walnuts intake in 13 trials and 61 trials.

Although there are plenty of studies suggesting the beneficial effects on nuts consumption (O'Neil, Keast et al. 2011), evidence of general nuts consumption on CV markers among Asian population health remains inadequate. One latest randomized controlled trial testing on an Asian Indian population indicated that a single intervention with pistachios exert beneficial effects on the cardio-metabolic profile such as TC, LDL-C, *hs*-CRP, FFAs, adiponectin and TNF- α (Mohan, Gayathri et al. 2018). Moreover, 6-week of interventions with mixed nuts exerted favourable effects on lipid parameters such as TC and non-HDL-C in Korean women (Lee, Nam et al. 2014). According to one latest Chinese systematic review (Xiao, Huang et al. 2017), only walnuts significantly improve endothelial function.

3.1.2 Ethnicity and cardiovascular health

The term 'ethnic groups' was referred by Social Research Council (ESRC) as 'people of the same race or nationality with a long-shared history and a distinct culture' and ESRC defined 'ethnicity' as the 'intangible quality, or sense of being, derived from that shared racial or cultural affiliation' (Leung and Stanner 2011). Nowadays, there are a rich mix of cultures and culturally diverse communities which are related to changes in society such as immigration in the UK (Leung and Stanner 2011). Evidence showed that Chinese immigration in the UK tend to show a relatively lower risk of CVD than white British, Pakistani and other white populations (Bansal, Fischbacher et al. 2013, Li and Ge 2015). A survey was launched in Newcastle upon Tyne in the UK which showed that Chinese males had a lower TC (5.1 versus 5.6 mmol/L, $p < 0.001$), lower LDL-C (3.2 versus 3.6 mmol/L, $p < 0.001$) and lower BMI (23.8 versus 26.1 kg/m², $p < 0.001$) than European men. Chinese women also had lower lipid levels (TC: 4.9 versus 5.4 mmol/l $p < 0.001$, LDL-C: 2.8 versus 3.1 mmol/L $p < 0.001$); and BMI values (23.5 versus 26.1 kg/m², $p < 0.001$) (Harland, Unwin et al. 1997).

However, the proportion of Asian people with a high risk CVDs was substantial at BMIs lower than the existing WHO cut-off point for overweight (≥ 25 kg/m²) (Barba, Cavalli-Sforza et al. 2004) and CVD mortality and morbidity in China were increasing persistent although Chinese adults were less likely to report a long-standing illnesses compared with the general population (Leung and Stanner 2011). Beside the fact that Asian and Caucasian population has different body composition, fat distribution and the associated metabolic

profiles due to genetics (Wulan, Westerterp et al. 2010), lifestyle factor, especially dietary factor such as nuts consumption, largely play a critical role in the ageing process of heart function.

Currently no study aims to research different effects of general nuts consumption in different ethnic groups – Asian (mostly Chinese) and non-Asian population (mostly Caucasians) as a systematic review and meta-analysis. To address this knowledge gap, the aim of this systematic review is to do a comprehensively quantitative evaluation of the literature and perform a meta-analysis by examining whether or not there is any changes particularly in markers of endothelial dysfunction (FMD), blood markers including cell adhesion molecules (sVCAM-1, sICAM-1), oxidative damage (oxidised-LDL), markers of inflammation (IL-6, IL-10, CRP) as well as lipid concentrations (TC, HDL-C, LDL-C, TG, VLDL-C, Apo A-I, Apo B) induced by a number of interventions with nuts consumption or nut-enhanced diets.

3.2 Subjects and methods

This project is a systematic review and meta-analysis of randomized controlled trials (RCTs). PROSPERO is an international database of prospectively registered systematic reviews in health and social care, welfare, public health, education, crime, justice, and international development, where there is a health-related outcome (<http://www.crd.york.ac.uk/PROSPERO/#index.php>). The motivations for registration are related to ensure transparency in the review process, helps counter publication bias by providing a permanent record of prospectively registered reviews, irrespective of whether they are eventually published or not. The protocol has been registered with PROSPERO, the International Prospective Register of Systematic Reviews, indexed under (Registration number **CRD 42018089055**).

This systematic review was undertaken in adherence with the established methods recommended by the Cochrane (Higgins and Green 2011) and the Centre for Reviews and Dissemination guidelines (Centre for Reviews and Dissemination 2009, Tacconelli 2010). This systematic review is also reported according to PRISMA guidelines (Moher, Liberati et al. 2009) (**Appendix B1**) as PRISMA concentrates on reporting of reviews evaluating randomized trials as well as reporting systemic reviews/ meta analyses of a wide range of researches, especially for assessments of interventions.

3.2.1 Search strategy and study inclusion criteria

A complete search of the interventional trials on nuts was undertaken through August 2017 in the following electronic databases: **MEDLINE** (beginning 1990 until August 2017) (<http://www.pubmed.gov>); **Scopus** (beginning 1966 until 2017) (website: <https://www.scopus.com/home.uri>); **Web of Science** (beginning 1991) (website: http://apps.webofknowledge.com/WOS_GeneralSearch_input.do?product=WOS&search_mode=GeneralSearch&SID=U2g4OpjuGuzA2LJM3qP&preferencesSaved=).

The search strategies as performed comprehensively and the following search term were covered: (“Cardiovascular risk factors” OR “Cardiovascular diseases”) AND (“endothelial function” OR “endothelial dysfunction” OR “flow-mediated dilation” OR “FMD” OR “arterial stiffness” OR “carotid intima-media thickness” OR “intercellular adhesion molecule-1” OR “ICAM-1” OR “vascular cell adhesion molecule-1” OR “VCAM-1” OR “e-selectin” OR “p-selectin” OR “dimethylarginine” OR “ADMA” OR “oxidized low density lipoprotein” OR “oxidized-LDL” OR “inflammation” OR “C-reactive protein” OR “CRP” OR “LDL cholesterol” OR “HDL cholesterol” OR “triglycerides” OR “total cholesterol”) AND (“walnuts” OR “nuts”) AND (“randomized controlled trial” OR “randomized” OR “clinical trial as topic” OR “placebo” OR “randomly” OR “trial”) NOT (“animal”). The systematic review registration is CRD42018089055.

If a site-specific dataset had been published more than once, most current publication was used. English language restrictions were imposed, and duplicate citations were removed. In addition, a manual search of references from primary or review articles was performed to identify additional relevant trials. Literature search was conducted independently by three authors (FL, JL, and HMC), with disagreements resolved by consensus. Furthermore, for each of the relevant abstracts, full publications were retrieved for evaluation on the basis of criteria established a priori.

3.2.2 Study selection

The selection of references throughout the search strategy and the data extraction was performed consistent with specific criteria which are delineated below/ the following inclusion criteria were defined prior to study selection process:

- a) Nutritional/dietary interventions: Interventions with evaluating the use of nuts (in any types or doses) versus a control or placebo group.

- b) Study design: Randomized controlled trials (RCTs) on human subjects with either parallel or crossover design – Crossover trials that reported data separately among different treatment periods were analysed and recorded as a parallel trial using data from the first period; Length of study: minimum intervention period of four days.
- c) Subjects: adults.
- d) No other supplementation.
- e) Assessment of the “outcome of interest”: **Markers of inflammation** (C-reactive protein, interleukin-6, TNF- α , adiponectin); **endothelial function/endothelial dysfunction** (intercellular adhesion molecule-1, vascular cell adhesion molecule-1, flow-mediated dilatation, pulse wave velocity, nitric oxide); **Blood lipids** (plasma TC, HDL-C, LDL-C, VLDL-C, TG, Apo B and Apo A-I); Serum markers of oxidative status (e.g., ox-LDL). Reporting data on at least one of the following endpoints: total cholesterol, LDL cholesterol, HDL cholesterol or triglycerides.
- f) Report of post-intervention mean values (or if not available, change from baseline values were used instead) with standard deviation (or basic data which allow to calculate these parameters, i.e., standard errors, 95% confidence interval, p-values).

Exclusion criteria mainly included:

- a) Studies that reported redundant results of the same RCT.
- b) Non-interventional studies and interventions not involving any kinds of nuts; or interventions combined interventions in which the effects of nuts cannot be singled out; or interventions are acute
- c) Outcomes: non-cardiovascular outcome measures.
- d) PREDIMED studies with the Mediterranean diet

Two investigators reviewed potentially relevant articles independently with discrepancies being resolved through consensus (JL, FL). Institutional review board approval was not necessary for this systematic review.

3.2.3 Data extraction and management /Strategy for data synthesis

The full text of studies meeting inclusion criteria was recovered and screened to determine eligibility by 2 reviewers (FL, JLG). Following assessment of methodological quality, an independent reviewer FL extracted data onto a purpose-designed data extraction form and summaries containing the most important results from each paper. Descriptive data extracted included: study design, year of publication, country of origin, randomization, duration and length of follow-up, design of RCTs (parallel or crossover), methods of analysis, completion rates; participant characteristics (population, settings of interventions, baseline characteristics, mean (range) age, gender distribution of study participants, sample size); outcome measures (dietary and/or nutritional intake, body mass index, CV biomarkers) were summarized in **Table 3.1**; the 2 reviewers confirmed all data entries. Description of nuts intervention and respective control. Study quality was assessed using the Cochrane risk of bias tool (Higgins and Green 2011, Cuevas-Ramos, Almeda-Valdes et al. 2013).

The primary outcomes of the analyses were changes in CV risk factors after nuts consumption. Measures included blood lipids (TC, LDL-C, and HDL-C, TG, VLDL-C, Apo B, Apo A-I, ox-LDL), assessment of endothelial function by FMD, PWV, SBP and DBP.

3.2.4 Quality assessment

An assessment of bias in the included manuscript was performed by the Cochrane criteria (Higgins JP and S 2008). The objects used for the assessment of manuscripts were including: acceptability of random sequence generation, distribution concealment, blinding of subjects, employees and consequence assessment, talking of drop-outs, selective outcome reporting, and additional possible causes of bias (Higgins and Green 2011).

3.2.5 Statistical analysis

The software Review Manager (RevMan Version 5.1 for Windows Copenhagen: The Nordic Cochrane Centre, The Cochrane Collaboration, 2011) was used to pool and analyse results from the individual studies. Pooled results are reported as mean differences with 95% CIs and with two-sided P-values. However, when variables (such as IL-6 or CRP, and ICAM-1) were reported by mean differences (MD) and a summary statistic for comparing effect sizes across studies were used. Subgroup analysis was undertaken to evaluate the difference of cardiovascular markers in two ethnicities based on the participants' country as well as

participants' ethnicity which are reported from studies included in our review. The subgroup of ethnicity was extracted from studies reporting ethnicity of their participants while the subgroup of country was extracted from all studies in our review. Because the country of published paper does not 100% represent the ethnicity of the participants, therefore in order to ensure the accuracy of the ethnicity of including participants, two subgroups were abstracted. It reported that studies originated from non-Asian regions are more than studies originate from Asian countries. In addition, meta-regression analysis was used to assess the association between cardiovascular markers with the amounts and the length of nuts consumption.

A random effects model accounting for inter-study variation was used, thereby minimizing potential bias due to methodological differences between studies. Multiple dietary intervention arms from three studies were included in the meta-analysis. Following previous guidance (Higgins and Green 2011) excessive weightings from "double counts" arising from the "shared" group (in this case, the control group) were controlled by splitting the sample size of the shared group into approximately equal smaller groups for the comparisons. In this analysis we sought to extract and analyse adjusted results from multivariate models, if reported in the studies.

Heterogeneity was evaluated using the I^2 statistic (Centre for Reviews and Dissemination 2009, Higgins and Green 2011). Levels of heterogeneity are commonly regarded as high when I^2 values are >70%. Publication bias was appraised by visually inspecting the funnel plot, and supplemented with calculations of the Egger's regression test (Egger, Davey Smith et al. 1997). Quality of studies was assessed using the Jadad system (Jadad, Moore et al. 1996).

3.3 Results

3.3.1 Flow of studies

The searches yielded 199 citations of which 89 were retrieved for complete review after de-duplication and results of the screening process through a manual reference search of primary and review articles are described in **Figure 3.1**. In total, 89 publications that met inclusion criteria were included in the present systematic review (**Table 3.1**) (Sabate, Fraser et al. 1993, Chisholm, Mann et al. 1998, Spiller, Gates et al. 1998, Kris-Etherton, Pearson et al. 1999, Curb, Wergowske et al. 2000, Rajaram, Burke et al. 2001, Iwamoto, Imaizumi et al. 2002, Sabate, Haddad et al. 2003, Hiraoka-Yamamoto, Ikeda et al. 2004, Ros, Nunez

et al. 2004, Tapsell, Gillen et al. 2004, Chisholm, Mc Auley et al. 2005, Tamizifar B 2005, Kocyigit, Koylu et al. 2006, Kurlandsky and Stote 2006, Schutte, Van Rooyen et al. 2006, Perez-Martinez, Lopez-Miranda et al. 2007, Sheridan, Cooper et al. 2007, Gebauer, West et al. 2008, Griel, Cao et al. 2008, Olmedilla-Alonso, Granado-Lorencio et al. 2008, Claesson, Holm et al. 2009, Rajaram, Haddad et al. 2009, Tapsell, Batterham et al. 2009, Brennan, Sweeney et al. 2010, Ghadimi Nouran, Kimiagar et al. 2010, Lopez-Uriarte, Nogues et al. 2010, Ma, Njike et al. 2010, Rajaram, Connell et al. 2010, Torabian, Haddad et al. 2010, West, Krick et al. 2010, Wien, Bleich et al. 2010, Wu, Pan et al. 2010, Casas-Agustench, López-Uriarte et al. 2011, Davidi, Reynolds et al. 2011, Din, Aftab et al. 2011, Li, Liu et al. 2011, Sola, Fito et al. 2011, Tey, Brown et al. 2011, Aronis, Vamvini et al. 2012, Chiang, Haddad et al. 2012, Foster, Shantz et al. 2012, Mohamedou, Tacha et al. 2012, Sola, Valls et al. 2012, West, Gebauer et al. 2012, Damasceno, Sala-Vila et al. 2013, Liu, Liu et al. 2013, Orem, Yucesan et al. 2013, Somerset, Graham et al. 2013, Tey, Gray et al. 2013, Bento, Cominetti et al. 2014, Burns-Whitmore, Haddad et al. 2014, Colpo, Vilanova et al. 2014, Gulati, Misra et al. 2014, Lee, Nam et al. 2014, Moreira Alves, Boroni Moreira et al. 2014, Parham, Heidari et al. 2014, Sauder, McCrea et al. 2014, Sweazea, Johnston et al. 2014, Berryman, West et al. 2015, Carvalho, Huguenin et al. 2015, Chen, Holbrook et al. 2015, Huguenin, Moreira et al. 2015, Jamshed, Sultan et al. 2015, Njike, Ayetey et al. 2015, Ruisinger, Gibson et al. 2015, Sauder, McCrea et al. 2015, Agebratt, Ström et al. 2016, Dhillon, Tan et al. 2016, Bamberger, Rossmeier et al. 2017, Lee, Berryman et al. 2017, Mah, Schulz et al. 2017, Zibaenezhad, Farhadi et al. 2017, de Souza, Gomes et al. 2018, Jenkins, Kendall et al. 2018, Jung, Chen et al. 2018, McKay, Eliasziw et al. 2018, Mohan, Gayathri et al. 2018) However, 91 studies did not meet the inclusion criteria and were excluded for the following reasons as shown in **Figure 3.1**.

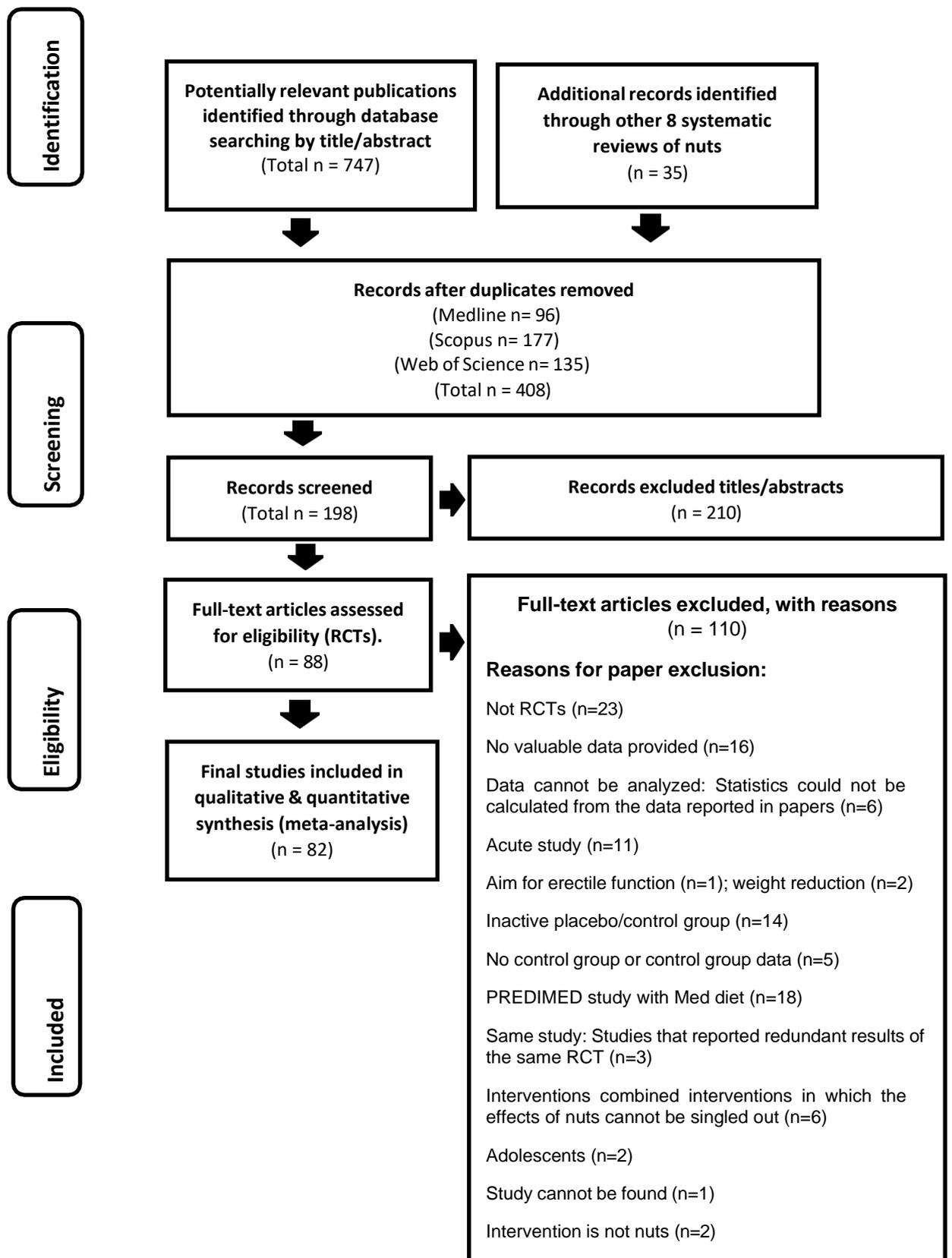


Fig. 3.1. PRISMA flow diagram of selection of studies on a variety of nuts consumption and CV risk factors.

3.3.2 Literature Search and Study Characteristics

From the overall pool of 88 RCTs that met the inclusion criteria (**Table 3.1**), six studies originated from East Asian ethnicity in total: 2 studies from Japan, 1 study from South Korean, two studies from Taiwan China and one study from Shanghai China. 77 studies originated from non-Asian ethnicity countries: 37 trials from USA, 6 trials from Spain, five articles from Canada and Brazil respectively, 4 trials from Australia and Iran respectively, 3 studies from UK and New Zealand respectively, 2 trials from India and Turkey respectively. Single study from Greece, Sweden, Pakistan, Morocco, South Africa and Italy respectively. In addition, 22 studies reported ethnicity of participants. three papers recruited 100% Caucasian while 14 trials had participants mostly constituting of Caucasian (white) population. One study recruited 100% Indians, 2 study recruited 100% Japanese, 2 study recruited 100% Chinese. 6 studies out of 88 RCTs are not quantitative synthesized into meta-analysis.

Table 3.1 present the general characteristics and results of the 82 included papers. Forty-seven studies used a randomized crossover design, and 35 studies are parallel design. The pooled study population meta-analyse included 3814 participants who were followed-up for approximately 14 weeks on average and the study duration varied between 4 days and 18 months. The mean ages of the samples in these studies ranged from 18 to 75 years and mean age is 47 years. Participants had existing disease conditions in 58.5% (48/82) of all randomized controlled trials (**Table 3.1**). **Table 3.1** shows a brief summary of different dose of nuts for different types of nuts in numbers of papers included in this review.

Overall, intervention of mixes of nuts includes a variety of nut types of cashews, peanuts, walnuts, almonds, pistachios, hazelnuts, Brazil nuts, macadamias, pine nuts and pecan nuts which were included in 82 studies. The control group consists of a variety of fruits or muffins or habitual diet or qualitative recommendations according to the American Heart Association dietary guidelines. According to **Table 3.3**, this meta-analysis includes most trials examined walnuts (25 studies) followed by pistachios (13 studies). The largest amount of nut consumption is pistachio and the average amount per day consumption reaches 76.6 grams. Daily consumption of pecans and cashews is also high, and they are 70 grams and 72.3 grams respectively. In addition, seven studies do not clarify the dose of nut intake.

3.3.3 Meta-analysis of studies consuming nuts

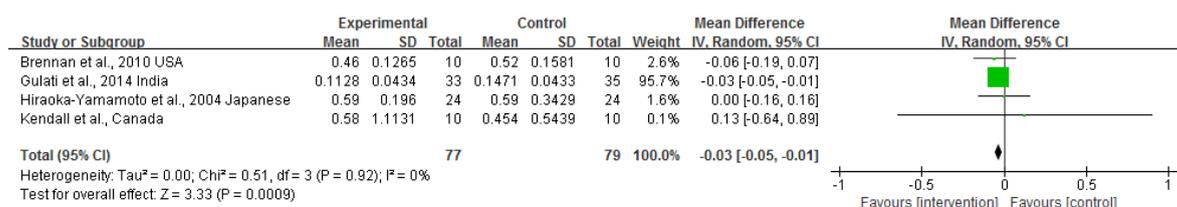
Meta-analysis of effects of nuts consumption was undertaken on 28 outcome parameters. Main biomarkers include blood lipids (TC, HDL-C, LDL-C, VLDL-C, Apo A-I and Apo B); inflammatory biomarkers (E-selectin, P-selectin, ICAM-1, VCAM-1); pro-inflammatory cytokines (CRP, hs-CRP, IL-1, IL-6, TNF-alpha); anti-inflammatory cytokines (adiponectin, IL-10); chemokines (MCP-1); oxidative stress biomarkers (oxidized LDL); markers of nitric oxide bioavailability (FMD); markers of measuring arterial stiffness (PWA, PWV); antioxidant

biomarkers (TAC); factors affecting endothelial function (nitric oxide) and blood pressure (SBP, DBP). However, only 8 outcomes were presented to be improved after nuts intake.

Forest plots present pooled mean differences with 95% confidence intervals (CI) for randomized controlled studies. For each study in each figure, the shaded square represents the point estimate of the intervention effect. The horizontal line joins the lower and upper limits of the 95% CI of these effects. The area of the shaded square reflects the relative weight of the study in the respective meta-analysis. The diamond at the bottom of the graph represents the pooled MD with the 95% CI for all study groups. Pooled estimates of effects size for all markers of positive results after nut consumption are summarized in **Table 3.2**.

4 papers representing 156 participants reported results for FFA (mmol/L). Overall, nuts interventions significantly reduced by 0.03 (95% CI: -0.05 to -0.01; $P=0.0009$; $I^2=0\%$) in comparison with the control groups; the levels of heterogeneity are low: $I^2 = 0\%$ (**Fig 3.2**).

Figure 3.2. Effects of nuts consumption on FFA (mmol/L).



Forest plot showing pooled mean differences with 95% confidence intervals (CI) for randomized controlled diets. For each study, the shaded square represents the point estimate of the intervention effect. The horizontal line joins the lower and upper limits of the 95% CI of these effects. The area of the shaded square reflects the relative weight of the study in the respective meta-analysis. The diamond at the bottom of the graph represents the pooled MD with the 95% CI for all study groups.

Markers of blood lipids cholesterol and apolipoproteins – TC, TG, HDL-C, LDL-C, VLDL-C and Apo B

71 studies representing 3670 participants assessed the impact of nuts on total cholesterol (TC) concentrations (**Fig 3.3**). Overall, nuts intake significantly reduced the level of TC by 7.19 mg/dL (95% CI: -9.66 to -4.72; $P < 0.00001$; $I^2=57\%$) in comparison with the control groups; the levels of heterogeneity are low: $I^2 = 57\%$ (**Fig 3.3**). A funnel plot of the mean differences in TC concentrations levels against standard error (SE) of all studies present slightly symmetry suggesting the absence of publication bias as shown in **Fig 3.10**.

Figure 3.3. Effects of nuts on blood lipids: TC (mg/dL).

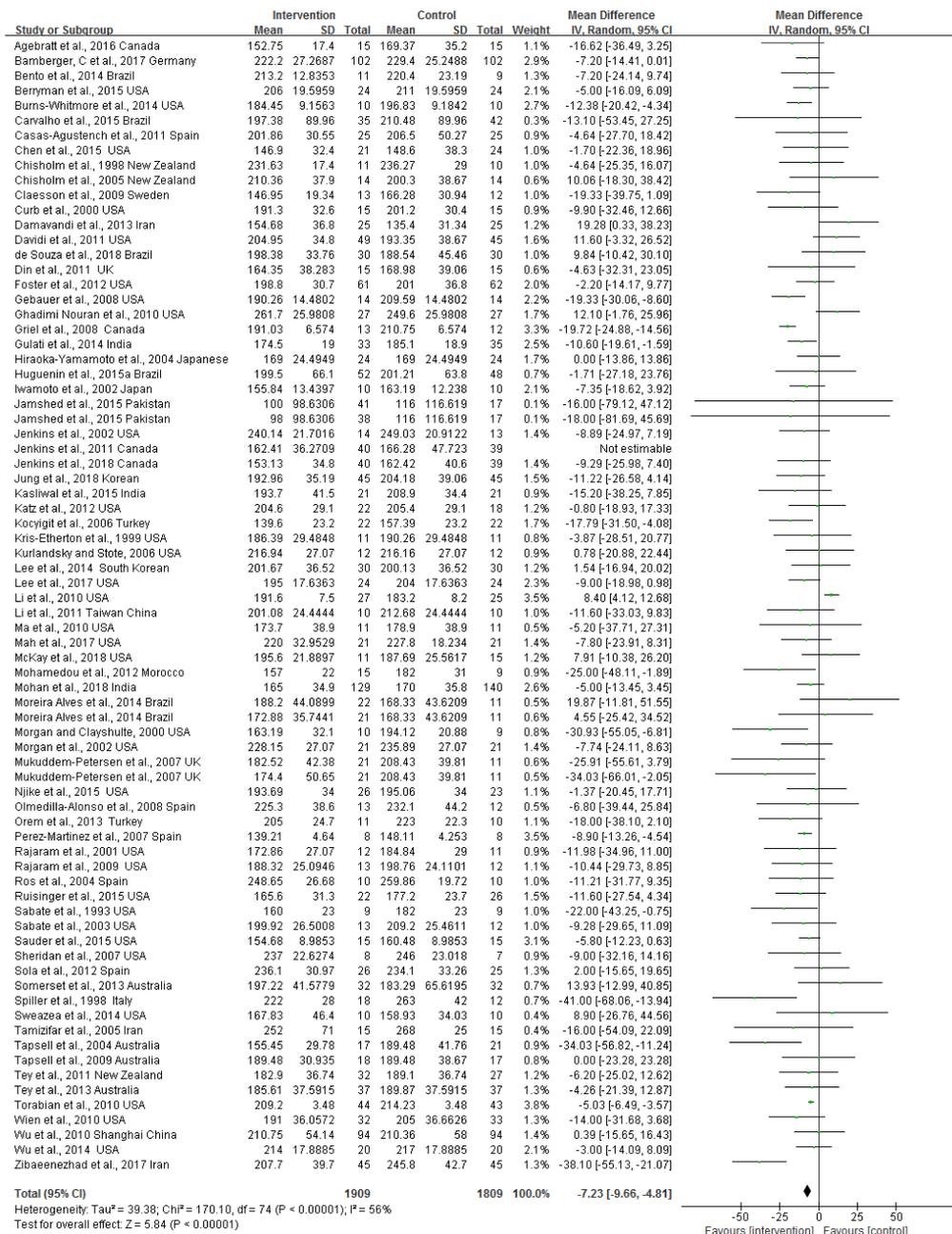
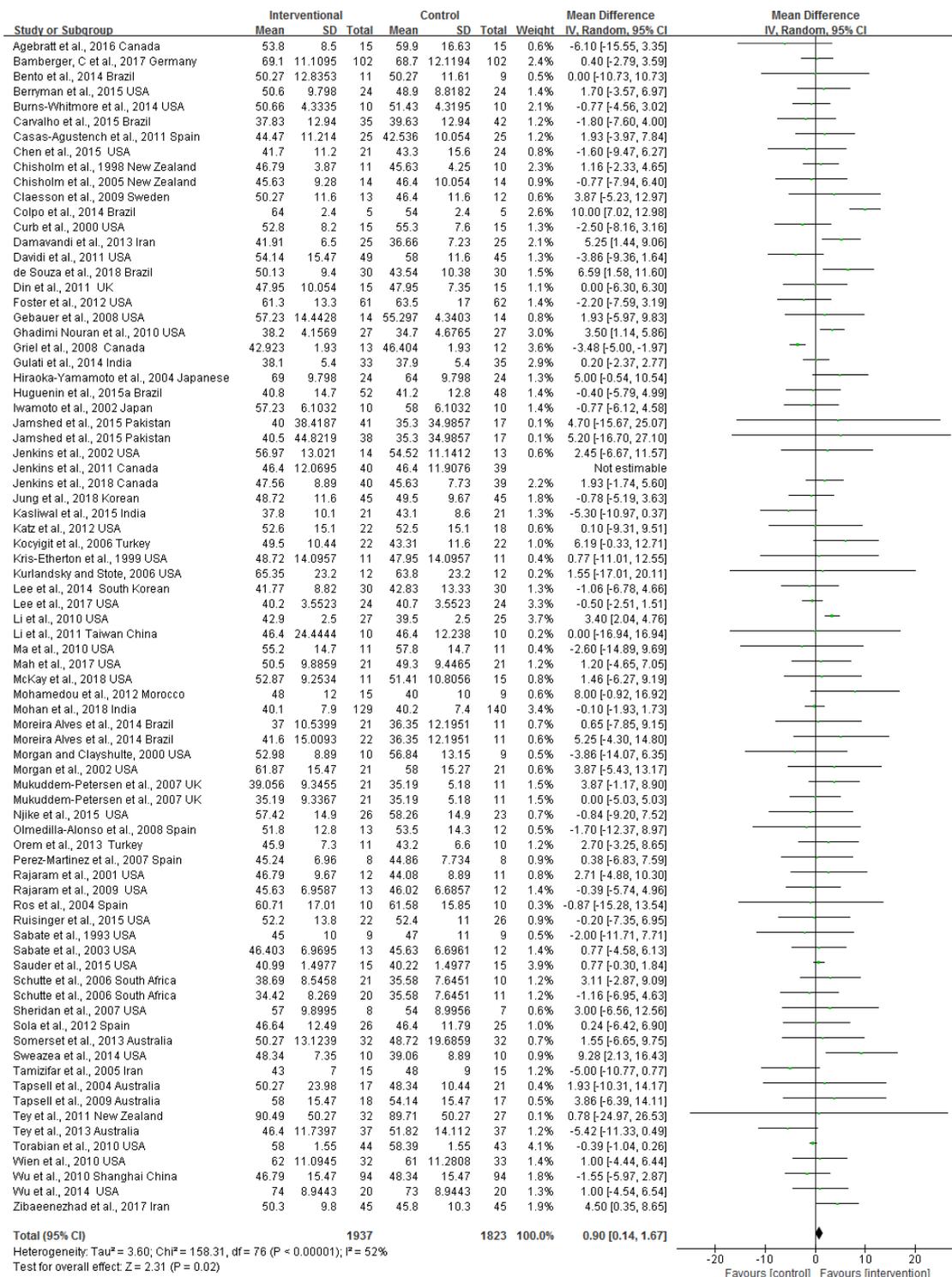


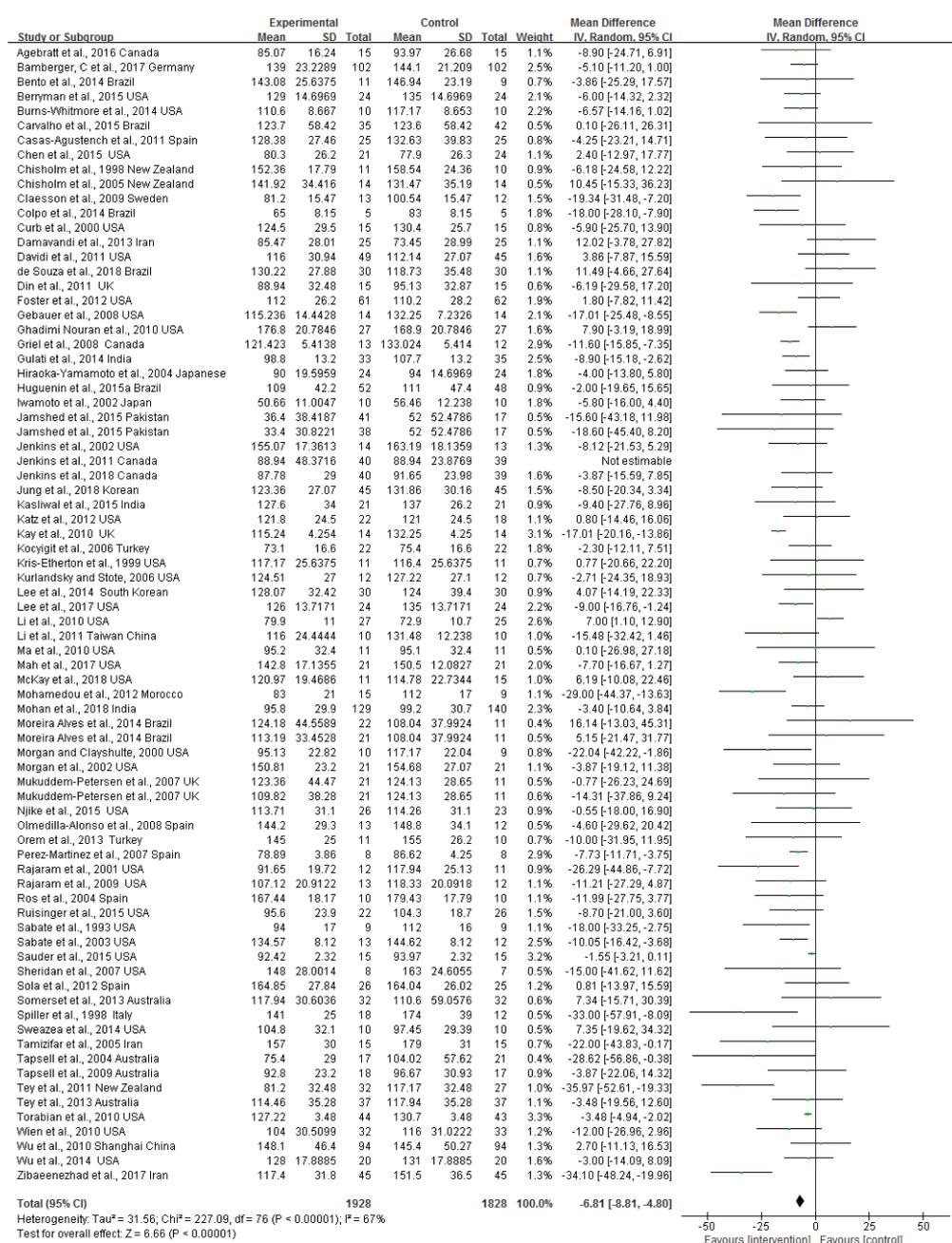
Figure 3.4. Effects of nut consumption on blood lipids: HDL-C (mg/dL).

67 studies, including 3063 participants, evaluated the impact of nuts consumption on HDL-C. Overall, nut consumption significantly increased HDL-C by 0.95 mg/dl (95% CI: 0.16 to 1.74; p=0.02). Heterogeneity levels assessed by the I² test were low at 52% (Figure 3.4). A funnel plot of the mean differences in HDL-C levels against SE of all studies indicates that slightly symmetry suggesting the absence of publication bias (Fig. 3.11).



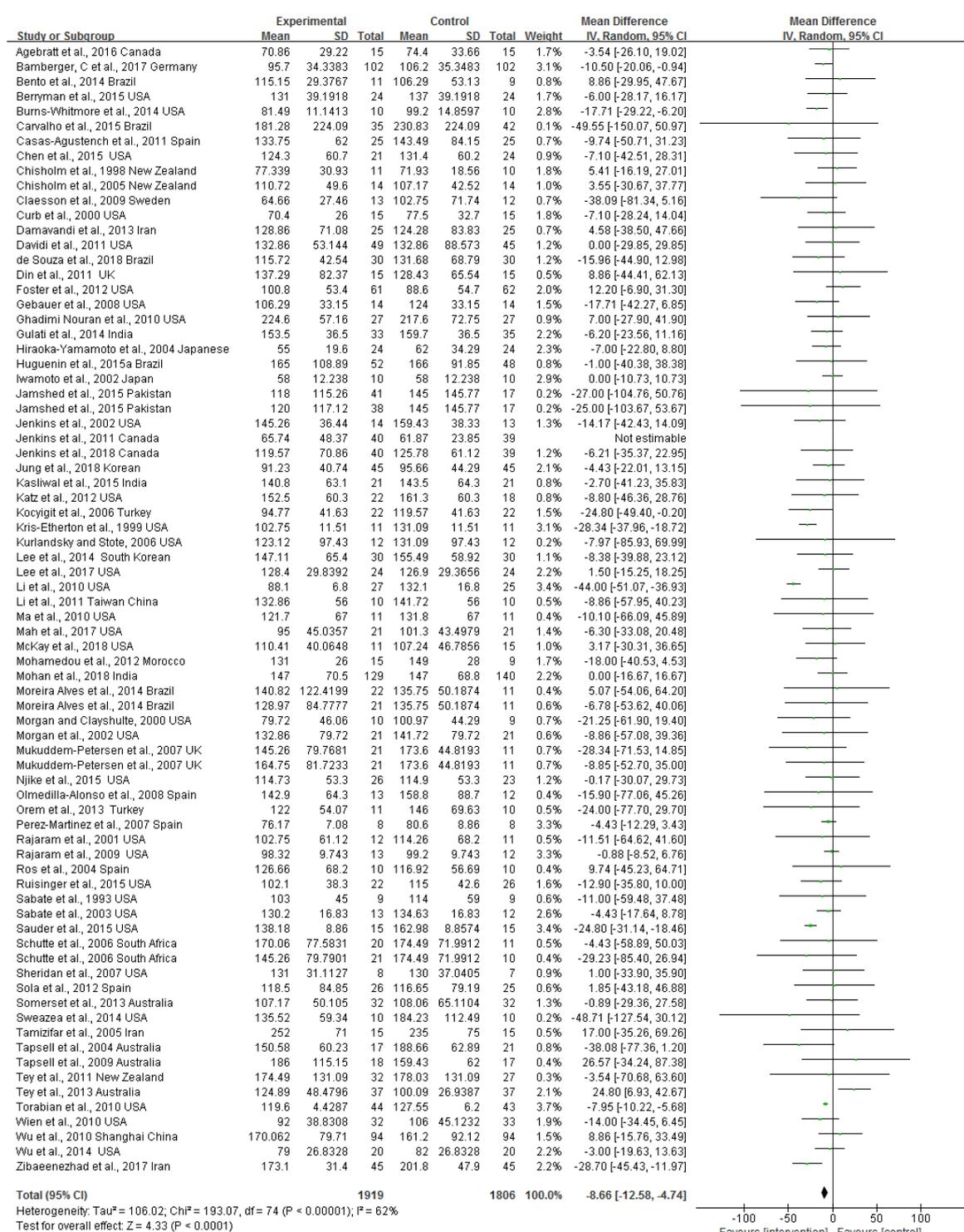
68 studies, including 3059 participants, evaluated the impact of nut consumption on LDL-cholesterol. Overall, nut consumption significantly reduced LDL-cholesterol by 7.21 mg/dl (95% CI -9.38 to -5.04; $p < 0.00001$). Heterogeneity levels assessed by the I^2 test were low at 68% (**Fig 3.5**). A funnel plot of the mean differences in LDL-C levels against standard error (SE) of all studies shows slightly asymmetry (**Fig 3.12**) suggesting that the presence of publication bias.

Figure 3.5. Effects of nut consumption on plasma LDL-C (mg/dl).



65 studies, including 3028 participants, evaluated the impact of nut consumption on triglycerides. Overall nut consumption significantly reduced triglycerides by 8.83 mmHg (95% CI -13.12 to -4.53; $p < 0.0001$). Heterogeneity levels assessed by the I^2 test were low at 64% (Fig. 3.6).

Figure 3.6. Effects of nut consumption on plasma triglycerides (mg/dl).



Ten studies, including 520 participants, evaluated the impact of nut consumption on plasma VLDL-cholesterol. Overall nut consumption significantly reduced plasma VLDL-cholesterol by 2.25 mg/dl (95% CI -3.74 to -0.77; $p = 0.003$). Heterogeneity levels assessed by the *I*² test were low at 0% (**Fig. 3.7**).

Figure 3.7. Meta-analysis of studies consuming nuts on plasma VLDL-C (mg/dl).

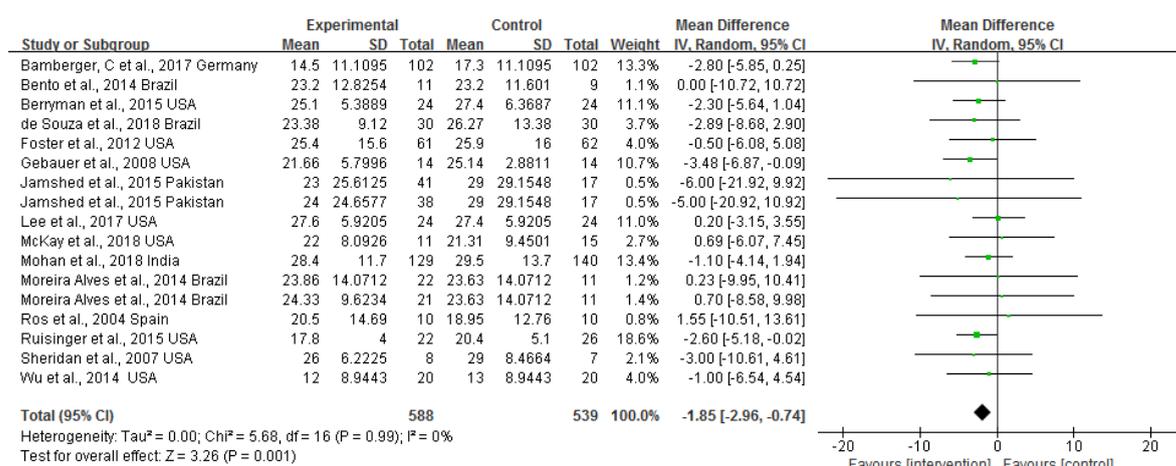
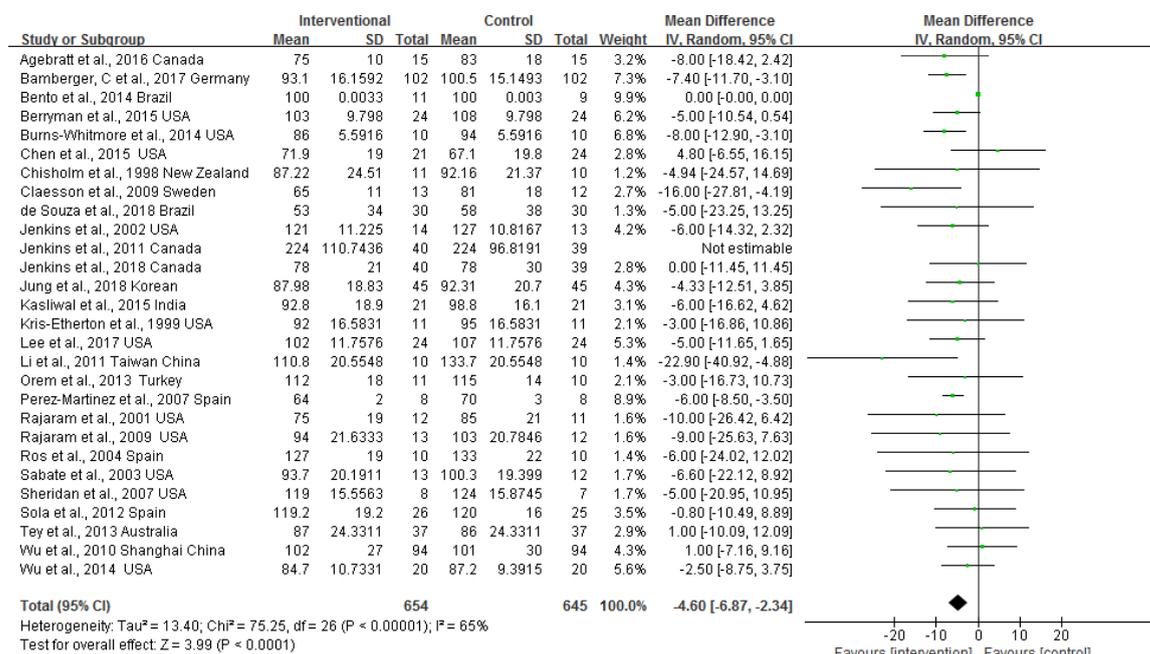


Figure 3.8. Meta-analysis of studies consuming nuts on Apo B (mg/dl).

23 studies, including 897 participants, evaluated the impact of nut consumption on Apo B. Overall, nut consumption significantly reduced Apo B by 4.47 mg/dL (95% CI -7.01 to -1.94; p = 0.0005). Heterogeneity levels assessed by the I² test were low at 0% (**Fig. 3.8**).



3.3.4 Subgroup analysis

Table 3.4 reports the difference of outcomes between ethnicity and country subgroups overall. Most outcomes comparisons showed no statistically significant difference between Asian and non-Asian groups in either subgroup of ethnicity or subgroup of country as P-values were $> .05$. Only two outcomes – lag time of LDL and TAC reported in the subgroup of country observed that there is statistical significance between Asian and non-Asian groups as p-values equal to 0.0004 and less than 0.0001 respectively and non-Asian population has a significant improvement on these two variables. However, only two studies reported lag time of LDL and three studies reported TAC.

Generally, non-Asian populations showed a tendency to have better improvement after nuts consumption in blood lipids - TC, HDL-C, LDL-C, and TG; FFA, adiponectin, IL-6, sICAM-1 and hs-CRP than Asian population although there is no statistically significant difference between Asian and non-Asian population as shown in **Table 3.4**. Evidence according to **Table 3.4** also indicated that nuts consumption showed better results on oxidized-LDL, SBP, Apo B, Apo A-I, CRP and sVCAM-1 in Asian individuals compared with non-Asian individuals in both subgroups of ethnicity and country although there is no statistically significant difference between Asian and non-Asian population.

In addition, FMD were only reported by non-Asian population based on country origin. DBP in non-Asian groups are consistently shown to decrease, but there are two studies originating from Asia reporting opposite results concerning DBP. VLDL-C was only reported by non-Asian studies. As for TNF-alpha cytokines, studies reporting non-Asian population showed inconsistent results due to inadequate studies from Asian population based on **Table 3.4**.

Meta-regression analysis (**Figure 3.15 ~ 20**) was performed by mixed effects regression (unrestricted maximum likelihood) to evaluate the relationship of the cardiovascular biomarkers with difference in means in amounts of mixed nuts consumption or the length of nuts consumption. The meta-regression analysis showed that greater dose of nuts may be associated with lower TG.

3.3.5 Study quality

Table 3.5 shows the methodologic quality of included studies in the meta-analysis. The Jadad rating scale was used to score the randomized trials from 1 to 5 points (Jadad, Moore et al. 1996, Clark, Wells et al. 1999). From a total of 82 papers, 14 studies were rated as 5 points, whereas 13 studies were rated as 4 points. 79 studies reported good feasibility and acceptability while one study reported bad feasibility and acceptability as no answers from participants to explain the reason why they did not follow (**Table 3.1**). The other one study reported nine dropouts due to poor compliance and another study reported compliance was weak as shown in **Table 3.5**.

61 studies described methods for monitoring or verifying patient compliance. Participant withdrawal or no dropouts from all 82 trials were addressed in 69 studies whereas 13 studies did not clarify dropout reasons. All 82 studies were randomized, but only 32 studies reported the appropriate method of random number generation whereas 60 studies did not report generation of random methods. Allocation concealment of treatment were addressed in only 6 trials.

3.3.6 Publication bias

The visual inspection of funnel plots (see Supplemental **Figure 3.9-14**) and Egger's tests provided mixed evidence for a publication bias. Egger's test and publication bias were completed by Comprehensive meta-analysis V2. Seven outcomes including TC, HDL-C, LDL-C, TG, VLDL-C, Apo B and FMD were reported into funnel plots and detected by using Egger's tests as these outcome parameters were reported by equal or more than 10 trials.

TC ($p=0.16368$), HDL-C ($p=0.31179$), LDL-C ($p=0.07333$), TG ($p=0.61396$) and VLDL-C ($p=0.21059$) presented no apparent bias as indicated by Egger's test. However, the plots of TC (**Fig. 3.10**), HDL-C (**Fig. 3.11**), LDL-C (**Fig. 3.12**), TG (**Fig. 3.13**) and VLDL-C (**Fig. 3.14**) were shown to be asymmetrical.

Egger's test observed that publication bias was presented in outcomes including Apo B ($P=0.00006$) as the plots of Apo B (**Fig. 3.14**) was shown to be asymmetrical.

3.4 Discussion

3.4.1. Principal findings

As far we are aware, this is the first meta-analysis of 82 randomized controlled trials involving 3814 participants to overall assess the effects after consuming 10 types of nuts on CV risk factors for averagely 14 weeks between Asian and non-Asian population. Meta-analysis showed that consuming variable doses of nuts (**Table 3.3**) significantly improved TC, HDL-C, LDL-C, TG and VLDL-C indexes with low heterogeneity in comparison with baseline or placebo. Outcomes such as FFA, Apo B and FMD levels also are significantly improved after nuts consumption compared with various inactive control diets (**Table 3.2**). More importantly, our findings highlight the different effects after consuming nuts between Asian and non-Asian ethnicity. Subgroup analysis based on ethnicity indicated that nuts consumption tended to exert a better result on oxidized-LDL, SBP, Apo A-I, Apo B, CRP, TNF- α and sVCAM-1 among the Asian group compared with the non-Asian population although such differences were not statistically significant. In comparison, non-Asian population achieved a better improvement

on biomarkers including blood lipids such as TC, HDL-C and TG, DBP, FFA, adiponectin, lag time of LDL, TAC, *hs*-CRP, sICAM-1 and IL-6 than Asian group.

The results of this systematic review and meta-analysis are in agreement with earlier reports such as a systematic reviews and meta-analysis of randomized controlled clinical trials. One study revealed that different types of nut consumption with different amounts exert no significant effect on CRP, IL6, adiponectin, IL10, and TNF- α (Mazidi, Rezaie et al. 2016). To evaluate endothelial function, one of the standard non-invasive tools is FMD, which is considered to reflect the local bioavailability of endothelium-derived vasoactive substances such as NO or endothelin-1 (Schroeder, Enderle et al. 1999). The systematic review suggested that walnuts consumption significantly improved endothelial function with duration less than 18 weeks, nut dose less than 67 gram per day or subjects with baseline FMD => 8.6% (Xiao, Huang et al. 2017). Correspondingly, a significant improvement in FMD with 0.79% of weighted mean differences after nuts intake was observed by a meta-analysis (Neale, Tapsell et al. 2017) and a meta-analysis of cohort studies also found a significant reduction in risk of cardiovascular events per 1% increase in FMD (Inaba, Chen et al. 2010). Decreased values of FMD are considered to be early markers of atherosclerosis as well as a predictor of future CVD events (Inaba, Chen et al. 2010).

According to study (Banel and Hu 2009), high-walnuts-enriched diets significantly decreased total and LDL-C for trials. Tree nuts overall intake were shown to lowers total cholesterol, LDL cholesterol, Apo B, and triglycerides (Del Gobbo, Falk et al. 2015). In addition, a pooled analysis of 25 trials indicated that blood lipids including TC, LDL-C and TG are improved after a daily consumption of 67g of nuts (Sabate, Oda et al. 2010). However, our finding of no significant effects on blood pressure and inflammatory biomarkers CRP, TNF- α , IL-6, ICAM-1, VCAM-1 or the anti-inflammatory biomarker adiponectin reflects the body of evidence available. Characteristics of the study sample or design of the dietary intervention may influence the ability to detect these effects.

3.4.2 Strengths and limitations

The strengths of these findings included that it used a systematic methodology following current guidelines for systematic reviews, including prospective registration and used the Jaded Score to evaluate the quality of evidence. The low levels of heterogeneity surrounding the results although each study had its own follow-up periods, inclusion criteria, basic health condition, carried periods of life, amounts of nuts and gender. High compliance of intervention diets and high retention rate were reported by most trials. Our systematic search makes it unlikely that large reports were missed, and error and bias were minimized by independent, duplicate decisions on study inclusion and data extraction. Effect sizes were standardized to a common dose. Furthermore, the duration of trials (average 14-week) was adequate to achieve changes and stabilization of lipid values (Kris-

Etherton and Dietschy 1997). We evaluated multiple CVD risk factors, including 28 outcomes; separately evaluated 9 types of nuts. These biomarkers were selected to reflect changes in disease progression and amelioration to explore mechanisms responsible for the favorable effects of nut consumption on CVD (Luo, Zhang et al. 2014) and other chronic conditions (Ibarrola-Jurado, Bullo et al. 2013, Afshin, Micha et al. 2014).

The identified trial populations were relatively diverse, including differences in age, sex, baseline comorbidity status, and background diets, enhancing the generalizability of our findings and make our findings may apply to a broad population. In addition, with the intention of identifying the different effects of nuts consumption between two ethnicities in all 82 randomized controlled trials, outcomes were compared by two subgroups, which are ethnicity and country. This improves the accuracy on identifying different effects of nuts consumption on ethnic groups.

Potential limitations should be considered when the results are interpreted. Compliance which was sometimes assessed by self-report in most included RCTs would cause overestimation of effects. There are limitations with meta-analysis as well. Primarily, a meta-analysis is limited by the methods, reported outcomes, and quality of the individual studies. When individual studies clearly describe their methods, fewer assumptions are made in data extraction and analysis when pooling multiple studies together. Nevertheless, larger numbers of trials in an analysis make it less likely that such errors could materially alter a final result.

Publication bias is another concern with meta-analyses. Negative or null findings fail to be submitted and/or accepted for publication. Despite extensive literature searches, meta-analysis can include only those studies that are published. Given that the pro-inflammatory cytokines such as TNF- α , IL-6 and CRP which characteristically induce endothelial cell activation also appeared unchanged, the lack of difference found for ICAM-1 and VCAM-1 is perhaps not surprising. There is also a concern that although final results show a significant improvement in lipids markers - LDL-C, VLDL-C and HDL-C. Apo A-I, which is the major protein in HDL-C, was not shown to increase but remained zero.

Our subgroup analysis addresses limitations as well. Although in our review we included as many outcomes as possible to pool and analyze, there is possibility that inadequate and imbalanced numbers of studies between Asian and non-Asian countries leading to no statistical significant difference in the majority of outcomes between Asian and non-Asian population. Two markers – Lag time of LDL and TAC were shown statistically significant difference between two ethnicities may be not representative because only two and three studies reported respectively.

3.4.3 Scientific analysis of findings

Frequent nut intake may improve biomarker outcomes via multiple mechanisms. Nuts are rich in unsaturated fatty acids and most contain substantial amounts of monounsaturated fatty acids (MUFA), which are important

contributors to the beneficial health effects namely prevention from the development of CVDs, lowering blood cholesterol and improvement of endothelial function (Ros and Mataix 2006). Specifically, Brazil nuts and pine nuts contain similar proportions of MUFAs and PUFAs especially linoleic acid, which is a polyunsaturated omega-6 fatty acid. Walnuts are rich in both LA and α -Linolenic acid (ALA), which is the plant n-3 fatty acid. Nuts that contain vitamin E include almonds, hazelnuts and peanuts. Walnuts, hazelnuts, pistachios, cashews, pecans, pine nuts, macadamias and Brazilian nuts all contained oleic and linoleic acid. Oleic acid is also found in almonds. Hazelnuts contain the most oleic acid, while walnuts contain the most linoleic acid. In addition, nuts are complex food matrices that also are sources of other bioactive compounds, namely: macronutrients, such as vegetable protein and fiber; other nutrients, such as potassium, calcium, magnesium, and tocopherols; and phytochemicals, such as phytosterols and phenolic compounds, among other bioactive compounds, such as resveratrol and arginine (Kris-Etherton, Yu-Poth et al. 1999). Phytosterols in nuts relate to the LDL-cholesterol response observed after nuts consumption (Escuriol, Cofan et al. 2009).

3.4.4 Implications for health and future research

The results of this review have important public health implications. A significant decline in vascular risk factors after average 14-week duration of a variety of nut consumption may potentially have important implications in primary prevention of atherosclerosis, cardiovascular diseases and cardiovascular mortality.

Reduced values of FMD are regarded to be early markers of atherosclerosis (Celermajer, Sorensen et al. 1992) as well as a predictor of future CVD events (Celermajer, Sorensen et al. 1992, Inaba, Chen et al. 2010). In relation with our findings on FMD, studies showed that increases in FMD of 1% increase, independently of confounding factors, are associated with reductions ranging from 10% to 13% in the risk of cardiovascular events (Inaba, Chen et al. 2010, Xu, Arora et al. 2014, Matsuzawa, Kwon et al. 2015).

Apolipoprotein A-I (Apo A-I) and Apolipoprotein B (Apo B) are two important components involved in lipid transport and in the processes causing atherosclerosis and CVD. Apo B, as the major protein in VLDL-C, is a stronger marker of cardiovascular risk for Caucasian (Sniderman, Islam et al. 2012) and for Chinese (Chien, Hsu et al. 2007) across varying age-groups and geographic regions than conventional lipids, lipoproteins markers (Schmidt and Bergstrom 2014). Therefore, a significant reduction in Apo B in both ethnicities supported healthy benefits with moderate nut intake in the present meta-analysis.

According to the study (Baigent, Keech et al. 2005), 1 mmol/L decrease in LDL-cholesterol is associated with a 23% reduction in myocardial infarction or coronary death and 12% reduction in all-cause mortality. Similarly, another study showed that 1 mmol/L decrease in LDL-cholesterol equates to a decrease in relative risk for stroke of 21.1% (Amarengo and Labreuche 2009). Consequently, in the present meta-analysis, there would be equally around a 4.4% reduction in myocardial infarction or coronary death, almost 2.3% decrease in all-cause mortality and approximately 4% reduction in risk of stroke after nut consumption.

3.4.5 Unanswered questions and future research

This work has observed the effect of well-established cardiovascular biomarkers such as blood lipids cholesterol, Apo B, FMD, with less evidence on inflammatory biomarkers (sICAM-1, sVCAM-1, PECAM-1 and E-selectin). ApoB/apoA-I ratio, pro inflammatory cytokines (CRP, TNF-alpha, IL-1 and IL-6), anti-inflammatory cytokines (IL-4, adiponectin, IL-10), chemokines (IL-8 and MCP-1) antioxidant biomarkers and oxidative stress biomarkers. These CV markers should be informative and researched in the near future. Further ongoing trials with standardized dosing and more Asian studies are also required to clarify the different effects nuts for the prevention of CVD among the Asian and non-Asian population.

3.5 Conclusions

In conclusion, this systematic review and meta-analysis of controlled trials observed that nuts consumption significantly lowers total cholesterol, HDL-cholesterol, LDL-cholesterol, triglycerides, VLDL-cholesterol, Apolipoprotein B (ApoB) and flow-mediated dilatation (FMD). Our findings also highlight the different effects of nuts intake between Asian and non-Asian population. This review observed no significant different effects after nuts consumption between different ethnicities but showed a valuable tendency of improvement on cardiovascular markers after nut intake among Asian and non-Asian population. Overall two ethnicities both achieved improvements on inflammatory biomarkers and lipids. Non-Asian population tends to obtain improvements on more CV markers than Asian population after nuts consumption. Specifically, non-Asian population seem to be more likely to improve better on blood lipids (TC, HDL-C, LDL-C, TG, VLDL-C), inflammatory biomarker (sICAM-1, IL-6) and anti-inflammatory cytokines (adiponectin) than Asian population after middle-term period of nuts intake while Asian group may also have the likelihood of presenting a better improvement on some biomarkers such as apolipoprotein (Apo A-I, Apo B), pro-inflammatory cytokines (CRP), inflammatory biomarker (sVCAM-1) and blood pressure (SBP). Although large-scale studies are needed to validate and replicate to test the nuts effects on different ethnicities, these results in the present review have potential public health implications and support the development of promising individualized nutritional strategies to tackle cardiovascular diseases.

Table 3.1. RCTs eligible for inclusion in the systematic review and meta-analysis in non-East Asians (mostly Caucasians).

(Author/ year) country of origin	Study design	Ethnicity (%)	Mean \pm SD/SE M or range Age	Sex (%male)	Mean BMI (kg/ m ²)	Health y status	Baseline sample size	Intervention length	Intervention	Dose & frequency	Control/ comparator	Retention rate	Feasibility & acceptability	Total Jadad score
Agebratt et al., 2016 Canada	RCT, blinded, parallel	N/A	23.5 \pm 3.7 y	60	22.3 \pm 1.9	Healthy	Fruit n=15; Nut n=15	4wks	Nut mixes	7 kCal /kg bodyweight daily	Bananas, apples citrus fruits, pears, melons, grapes, mangos, kiwis, or persimmons with less than 1% each from pineapples or plums	100	Good	4
Aronis et al., 2012 Greece	RCT, double- blinded, crossover	Caucasian	58 \pm 2.5y	60	36.6 \pm 1.7	Obese with metabolic syndrome (MS)	Total n=15	4d	Walnuts incorporated into a liquid meal	48g/d	Iso-caloric diet without walnuts	100	Good	4
Bamberger, C et al., 2017 Germany	RCT, crossover	Caucasian	63 \pm 7y	31	25.1 \pm 4.0	Healthy (non- smoking and post- menopausal)	Walnut n=102; Control n=102	8wks	Shelled walnuts	43g/d	Nut-free Western- type diet	95.1	Good	5

Bento et al., 2014 Brazil	RCT, crossover	N/A	34.9±2.7y	40	18.5±29.99 (18.9-27.8)	Mildly hypercholesterolemic	Total n=25	6wks	Baru almonds with usual diet	20g/d	One corn starch capsule daily	80	Good	4
Berryman et al., 2015 USA	RCT, crossover	White:94; Black:2; Asian:4	49.9±9.4y	46	26.2±2.8	Healthy	Total n=61	6wks	Unsalted, whole, natural almonds with skins	42.5g/d	Identical diet with an isocaloric muffin daily (no almonds)	78.7	Good	5
Brennan et al., 2010 USA	RCT, double-blinded, crossover	White:75; African American:21	59.0±SE M2.0y	50	37.0±SE M1.4	MS	Total n=20	4d(0.57wks)	Walnuts with liquid meal	48 g/d	32 g safflower oil	75	Good	4
Burns-Whitmore et al., 2014 USA	RCT, crossover, 3x3 latinsquare	White:50; Latino:25; Asian:10; Other: 15	38±3y	20	23±1	Healthy	Total n=26	6wks	Walnuts with habitual diet	24.3/d	Standard egg	77	Good	4
Carvalho et al., 2015 Brazil	RCT, double-blinded, parallel	N/A	60.1±10.3y	55.8	29.54±5.60	Dyslipidemia and hypertension	Brazil nut n=44; Placebo n=45	90d (12.9wks)	Defatted Brazil nut flour with diet	13g/d	Artificially flavored dyed cassava flour with diet	86.5	Good.	5
Casas-Agustench et al., 2011 Spain	RCT, parallel	N/A	51.8±8.4y(50y or >50y)	56	30.8±3.05	Healthy	Nut n=27; Control n=25	12wks	Mixed raw unpeeled nuts (walnuts, almonds and hazelnuts) with diet	30g/d	Diet rich in vegetables and fruits without nuts	96.2	Good	4

Chen et al., 2015 USA	RCT, crossover	N/A	61.8±8.6y (45-77y)	40	30.2±5.1 (20-41)	Coronary artery disease (CAD)	NCEP n=25; Almond n=22	6wks	Almonds diet	85g/d	NCEP Step 1 diet without nuts	95.74	Good	5
Chiang et al., 2012 USA	RCT, single-blinded, crossover	N/A	33y (23-65y)	56	24.8 (18.7-36.6)	Healthy	Total n=27	4wks	Walnuts diet	36.4g/d	Control diet	92.6	Good	3
Chisholm et al., 1998 New Zealand	RCT, crossover	N/A	< 65y	100	N/A	Hyperlipidaemia	Total n=21	4wks	Walnuts diet	78g/d	Low fat diet	76.2	Good	4
Chisholm et al., 2005 New Zealand	RCT, crossover	N/A	48.3±10.3y	17.9	26.9±3.2	Healthy	Total n=28	6wks	Nuts with low saturated fat diet	30g/d	Cereal with canola oil	100	Good	4
Claesson et al., 2009 Sweden	RCT, parallel	N/A	23.4±2.7y	42.3	22.2±1.7	Healthy	Total n=26	2wks	Roasted and salted peanuts	20kcal/kg	Candy (no chocolate or liquorice)	96.2	Good	4
Colpo et al., 2014 Brazil	RCT, crossover	N/A	24.7±3.4y (23-34y)	60	25±2.55	Healthy	Total n=10	4wks	Brazil nuts	50g/d	No Brazil nuts	100	Good	5
Curb et al., 2000 USA	RCT, blinded, crossover	White:53; Asian-Pacific Islanders :37; black:10	36.7y(18-53y)	47.1	24±2.4	Healthy	Total n=30	4wks	Macadamia nut diet	N/A	Typical American diet	88.2	Good	5

Damavandi et al., 2013 Iran	RCT, parallel	N/A	55.7±7.7y	32	≤35	T2DM	Hazelnut n=25; Control n=25	8wks	Raw, unsalted hazelnuts	29g/d	No hazelnuts	96	Good	4
Davidi et al., 2011 USA	RCT, parallel	N/A	53.7±14.4y	21.3	33.7±5.3	Healthy	Nut n=49; Control n=45	8wks	Nut snack bars	80g/d	Habitual ad libitum diet	100	Good	5
de Souza, et al., 2018 Brazil	RCT, parallel	N/A	20-59y	0	32.94±4.52	Overweight or obese	BAED n=30; BAFD n=30	8wks	Roasted baru almonds (BAED)	20g/d	Baru almond free diet (BAFD)	76.7	Good	5
Dhillon et al., 2016 USA	RCT, parallel	N/A	33±13y		32.7±3.7	Overweight or Obese	Almond n=43; NFD n=43	12wks	Almond with energy restricted diet	15% energy from almonds	Nut-free energy restricted diet	91.9	Good	4
Din et al., 2011 UK	RCT, single-blinded, crossover	Caucasia n:73.3; Indian Asian:16.7; East Asian:10	23±3y	100	24.5±2.3	Healthy	Total n=30	4wks	Walnut diet	15g/d	No walnuts	100	Good	5
Foster et al., 2012 USA	RCT, parallel	White:53.7; Black:39; Hispanic:2.4; Asian:0; Other:6.5	46.9±12.5y	8.94	33.95±3.6	Overweight and obese	Almond n=61; Nut-free n=62	72wks	Almond diet	28g/d	Nut-free diet	100	Good	5
Gebauer et al., 2008 USA	RCT, crossover	N/A	48±SEM 1.5y(35-61y)	35.7	26.8±SEM 0.7 (21-34)	Hypercholesterolaemia	Total n=28	4wks	Pistachio with lower-fat diet	94.5g/d (63-126)	Lower-fat diet without pistachios	100	Good	4

Ghadimi Nouran et al., 2010 USA	RCT, crossover	N/A	43±SEM 1.3y	100	27.5±SEM 0.5	Hypercholesterolaemia	Total n=60	4wks	Peanuts with habitual diet	77g/d	Habitual diet without peanuts	90	Good	4
Griel et al., 2008 Canada	RCT, crossover	N/A	50.2±8.4y(25-65y)	40	26.3±3.3(22-35)	Healthy	Total n=25	5wks	Macadamia nut diet	42.5g/d	Average American diet (AAD)	96	Good	4
Gulati et al., 2014 India	RCT, parallel	Indian	42.5±8.2y	54.4	30.9±7.5	MS	Pistachio n=33; Control n=35	24wks	Unsalted pistachio with healthy diet	49g/d	Healthy diet without pistachio	88.2	Good	4
Huguenin et al., 2015a Brazil	RCT, double-blinded, crossover	N/A	62.1±9.3y	51.6	N/A	Hypertension & dyslipidemia; Obese or overweight	Brazil nut n=64; Placebo n=61	12wks	Granulated Brazil nuts diet	13g/d	Flavored cassava flour	72.8	Good	5
Jamshed et al., 2015 Pakistan	RCT, parallel	N/A	60±1.1y(32-86y)	75.3	N/A	CAD	NI n=50; PA n=50; AA n=50	12wks	Pakistani almonds ; American almonds	10g/d	Without almonds	75.33	Good	5
Jenkins et al., 2002 USA	RCT, crossover	N/A	64±9y(48-86y)	55.6	25.7±3.0(20.5-31.5)	Healthy hyperlipidemia or postmenopausal	Total n=43	4 wks	Whole raw unblanched almonds	73±3g/d	Muffins (147±6g/d)	62.8	Good	4

Jenkins, et al., 2018 Canada	RCT, blinded, parallel	Europea n:52; India: 29; Eastern:9 ; African:8 Hispanic: 1 Native American :1	62±9.4y	65.8	29.2±4.35	T2DM	Nut n=40; Muffin n=39	12wks	Mixed nuts	75g/d	188 g/d muffin diet	89.8	Good	4
Kasliwal et al., 2015 India	RCT, parallel, open-label	N/A	25-60y	82.1	26.95±3.8	Mild dyslipidemia	LSM n=27; LSM pistachios n=29	12wks	Pistachios with lifestyle modification (LSM)	80g/d	LSM without pistachios	75	Good	4
Katz et al., 2012 USA	RCT, crossover, single-blinded	N/A	57.4±11.9y(30-75y)	39.1	>25	MS	Walnut n=23; Ad libitum n=23	8wks	Shelled, unroasted English walnuts-enriched ad libitum diet	56g/d	Ad libitum diet without walnuts	87	Good	4
Kay et al., 2010 UK	RCT, crossover	N/A	35-61y	35.7	26.8±0.7	Healthy, nonsmoking	Total n=28	4wks	Pistachios with lower-fat diet	94.5g/d (63-126)	Lower-fat control diet without pistachios	96.4	Good	4
Kendall et al., 2014 Canada	RCT, crossover	N/A	54±8y	40	37.5±7.9	MS	Total n=20	>5-10 wks	Pistachios and white bread	85g/d	White bread 50g	100	Good	4
Kocyigit et al., 2006 Turkey	RCT, parallel	N/A	33.1±6.95y	54.5	24.4±6	Healthy	Total n=44	3wks	Pistachio diet	70g/d (65-75)	Regular diet	100	Good	4

Kris-Etherton et al., 1999 USA	RCT, double-blinded, crossover	N/A	21-54y (x:-34 y)	40.9	20-27	Healthy	Total n=22	24d(3.43wks)	Peanuts and peanut butter	N/A	American Heart Association/ National Cholesterol Education Program Step II diet	100	Good	4
Kurlandsky and Stote, 2006 USA	RCT, parallel	N/A	46.6±9y (22-65y)	0	25.7±3.8	Healthy	Almond n=12; Control n=12	6wks	Almonds with diet	60g/d	Control diet without nuts	83.3	Good	4
Lee et al., 2017 USA	RCT, crossover	N/A	46.3±1.8y(30-70y)	58.1	29.6±0.5(25-40)	Overweight and obese	Total n=48	4wks	Almond diet	42.5g/d	AAD	65	Good	5
Li et al., 2010 USA	RCT, parallel	Asian:4.3 ; Black:20; Caucasian:47.1; Hispanic: 18.6; Other:11.4	46.4±SEM 2.2y	Pistachio : 24.3; Pretzel: 14.8	30.5±SEM 0.4	Obese	Pistachio n=36; Pretzel n=34	12wks	Salted pistachios	53g/d	Salted pretzels (56g/d)	74.3	Good	4
Lopez-Uriarte et al., 2010 Spain	RCT, parallel	N/A	51.8±8.4y(18-65y)	56	30.8	Healthy	Total n=52	12wks	Mixed raw nuts with skin	30g/d	The American Heart Association diet	96.2	Good	4
Ma et al., 2010 USA	RCT, single-blinded, crossover	N/A	58.1±9.2y	41.7	32.5±5.0	T2DM	Walnut n=12; Ad libitum n=12	8wks	Walnuts with an ad libitum diet	56g/d	Ad libitum diet without walnuts	87.5	Good	5

Mah et al., 2017 USA	RCT, crossover	White:82.4; Black or African American :11.8; Asian or Pacific Islander: 2; Other:3.9	55.7±SE M1.42y (21-73 y)	39.2	26.9±SE M0.39	Healthy	Total n=51	4wks	Cashews with typical American diets	46g/d (28-64)	Baked potato chips within a weight-maintenance diet (32-64 g/d)	82.4	Good	5
McKay, et al., 2018 USA	RCT, blinded, crossover	N/A	62.7±SE M2y	81	28.9±SE M0.8 (25-35)	Overweight or obese with central adiposity	Pecan n=11; Control n=15	4wks	Pecan diet	42.5g/d	AAD	100	Good	5
Mohamedou et al., 2012 Morocco	RCT, parallel	N/A	48±11.6y	29	23.6±3.4	Dyslipidemia	Argan n=15; Control n=9	3wks	Argan oil with toasted bread	25ml/d	Butter (20g/d)	46.2	Good	4
Moreira Alves et al., 2014 Brazil	RCT, parallel	N/A	18-50y	100	29.8±2.3 (26-35)	Overweight or obese	CVP n=24; HOP n=27; CT n=25	4wks	Conventional peanuts (CVP); High-Oleic Peanuts (HOP)	56g/d	Control diet without peanuts	85.50	Good	4
Morgan et al., 2002 USA	RCT, crossover, open-label	Caucasian:81; African-American :19	55.7±11.8y	40.5	27.7±5.8	Hypercholesterolemia (borderline high TC)	Total n=49	6wks	Walnuts diet	64g/d	Usual diet	85.7	Good	4
Morgan and Clayshulte, 2000 USA	RCT, parallel	N/A	45±10y	21.05	24±4.5	Healthy	Total n=23	8wks	Pecans	68g/d	No nuts	82.6	Good	4

Mohan et al., 2018 India	RCT, parallel	Indian	51±9.3y	55	26.0±3.4	T2DM	Cashew n=129 Control n=140	12wks	Unsalted, raw, broken cashew with diabetic diet	30g/d	Diabetic diet	90	Good	4
Mukuddem-Petersen et al., 2007 UK	RCT, parallel	Caucasian	45±10y	45.3	35.2	MS	Total n=68	8wks	Walnuts diet; Cashew nuts diet	85.5g/d (63-108)	Diet without nuts or nut-based ingredients	94.1	Good	5
Nieman et al., 2014 USA	RCT, crossover	N/A	38.0±1.6y(27-49y)	100	H(m):1.81±SEM0.02; W(kg):76.8±SEM2.3	Healthy (trained cyclists)	Total n=20	2wks	Pistachio	85g/d	Water with no pistachio	95	Good	5
Njike et al., 2015 USA	RCT, parallel	N/A	53.3±11.1y(25-75y)	25	30.2±4.1	Diabetes and non-smoker	Walnut n=28; Control n=28	6wks	Walnut ad libitum diet	56g/d	Ad libitum diet without walnuts	87.5	Good	5
Olmedilla-Alonso et al., 2008 Spain	RCT, crossover, unblinded	N/A	54.4±8.1y	60	30.0±3.8	Healthy	Total n=25	5wks	Walnuts with steak	19.4g/d	Restructured steak without walnuts	100	Good	4
Orem et al., 2013 Turkey	RCT, crossover	N/A	44.6±10.4y	85.7	27.15±3.07	Hypercholesterolemia	Total n=21	4wks	Natural or raw hazelnut diet	67.5g/d(49-86)	National Cholesterol Education Program adult treatment panel (ATP) III step 2 diet	100	Good	4

Parham et al., 2014 Iran	RCT, double-blinded, crossover	N/A	51.5±10.5y	24.4	31.2±5.31	T2DM	Total n=48	12wks	Pistachios	50g/d	Usual diet without pistachios	91.7	Good	5
Perez-Martinez et al., 2007 Spain	RCT, crossover	N/A	N/A (medical students)	100	N/A	Healthy	Total n=16	4wks	Walnuts (<i>Juglans regia</i> L.) with high-CHO enriched in vegetable n-3 FA diet	N/A	Typical western diet rich in SFA	100	Good	4
Rajaram et al., 2001 USA	RCT, single-blinded, crossover	Caucasia n:48; Asian:26; Hispanic: 17; African-American :8.7	25±55y	61	N/A	Healthy	Total n=24	4wks	Pecan diet	72g/d	Step I diet	100	Good	5
Rajaram et al., 2010 USA	RCT, single-blinded, crossover	N/A	40.9±SE M12.8y (20-60y)	56	>30	Healthy	Total n=27	4wks	Almond diet	68g/d	Cholesterol-lowering control diet without nuts	92.6	Good	5
Rajaram et al., 2009 USA	RCT, crossover (3 * 3 Latin square)	N/A	23-65y	56	18.7-36.6 (24.8)	Mildly hyperlipidemic	Total n=27	4wks	Walnut diet	42.5g/d	Control diet without nuts	92.6	Good	5
Ros et al., 2004 Spain	RCT, crossover	N/A	25-75y	N/A	N/A	Non-smoking, asymptomatic, hypercholesterolemia	Total n=21	4wks	Walnuts diet	52.5g/d	Med-diet	95.2	Good	4

Ruisinger et al., 2015 USA	RCT, parallel	N/A	59.7±6.1 y (18-78 y)	50	29.2±4.35	Healthy	Almond n=22; Control n=26	4wks	Almonds with ATP-III TLC Diet counseling	100g/d	ATP-III TLC Diet with no almond	96	Good	4
Sabate et al., 1993 USA	RCT, crossover, single-blinded	Asian:17 White:83	30y	100	18.7-30.6 (23.8)	Healthy	Total n=18	4wks	Walnut diet	84g/d	Identical reference diet	94.7	Good	5
Sabate et al., 2003 USA	RCT, crossover (3*3 Latin-square)	White:40; Hispanic:28; Asian:20; African American:12	41±13y(20-60 y)	56	>30	Healthy	Total n=27	4wks	Almond diet	84g/d	Step I diet	92.6	Good	4
Sauder et al., 2014 USA	RCT, crossover	N/A	56.1±7.8 y(40-74y)	50	31.2±6.1	T2DM	Total n=34	4wks	Pistachios with moderate-fat diet	93.5g/d (59-128)	Low-fat or fat-free snacks (i.e., pretzels, string cheese, etc.)	88.2	Good	4
Sauder et al., 2015 USA	RCT, crossover	N/A	56.1±7.8 y(40-74y)	50	31.2±3.1	T2DM	Total n=34	4wks	Pistachio with moderate-fat diet	93.5g/d (59-128)	Low-fat or fat-free snacks (i.e., pretzels, string cheese, etc.)	88.2	Good	4
Schutte et al., 2006 South Africa	RCT, parallel	N/A	21-65y	42.9	N/A	MS	Walnut n=20; Cashew n=21; Control n=21	8wks	Unsalted cashew nuts diet; Walnut diet	85.5g/d (63-108)	Control diet	100	Good	5

Sheridan et al., 2007 USA	RCT, crossover	N/A	60±SEM 3y	73	28±SEM 0.9	Hypercholesterolemia (cholesterol >210 mg/dl)	Total n=15	4wks	Pistachio	71g/d (57-85)	Regular diet	100	Good	4
Sola et al., 2012 Spain	RCT, double-blinded, parallel	N/A	53.3±10(43-65 y)	N/A	< 35	Pre-hypertensive, stage-1 hypertension and hypercholesterolemia	Cocoa A =28; Hazelnut n=28	4wks	Hazelnut with cocoa cream	30g/d	Cocoa cream	91.10	Good	5
Somerset et al., 2013 Australia	RCT, crossover	N/A	26-55y	60	34.5±SEM1	Overweight	Total n=64	10wks	Macadamia nuts diet	50% total fat	Usual diet	100	Good	5
Spiller et al., 1998 Italy	RCT, parallel	N/A	53±10y	26.7	66±13kg(weight)	Hyperlipidemia	Almond n=18; Control n=12	4wks	Almond diet	100g/d	Step I diet	100	Good	4
Sweazea et al., 2014 USA	RCT, parallel	N/A	25-75 y	42.8	35.35±8.3	T2DM>6 mo	Almond n=12; Control n=12	12wks	Almonds diet	43g/d	Usual diet	87.5	Good	4
Tamizifar B., 2005 Iran	RCT, single blinded, crossover	N/A	56±6.1y(48-82y)	56.7	24.1±4.5(17.5-36.1)	Hyperlipidemia	Total n=35	8wks	Almond powder with NCEP step 1 diet	25g/d	NCEP step 1 diet	85.71	Good	4

Tapsell et al., 2009 Australia	RCT, parallel	N/A	54±8.7y	N/A	33.1±4.2	T2DM	Walnut n=26, Control n=24	48wks	Walnuts diet	30g/d	Low-fat isocaloric diet	70	Weakening	5
Tapsell et al., 2004 Australia	RCT, parallel	N/A	59.3±8.1y	63.2	29.97±3.23	T2DM	Walnuts n=17; Control n=21	24wks	Walnuts diet	30g/d	Usual diet	94.7	Good	4
Tey et al., 2011 New Zealand	RCT, parallel	N/A	37.4±14.0y	47	23.8±3.0	Healthy	Hazelnut n=32; Control n=31	12wks	Hazelnuts with chocolate and potato crisps	42g/d	Usual diet	85.71	Good	5
Tey et al., 2013 Australia	RCT, parallel	N/A	42.5±12.4y	43	30.6±5.1	Overweight and obese	Nuts n=37; Control n=38	12wks	Hazelnuts	60g/d	No nuts	98.7	Good	4
Torabian et al., 2010 USA	RCT, crossover	N/A	54±10.2y (30-72y)	43.7	26.5±3.3	Healthy, non-smoking	Total n=87	24wks	Walnut diet	46g/d	Habitual diet	100	Good	4
West et al., 2012 USA	RCT, crossover	N/A	N/A	35.7	21-35	Dyslipidemia	Total n=28	2wks	Pistachios diet	20% of total energy from pistachios	Typical Western diet	100	Good	4
West et al., 2010 Canada	RCT, crossover	N/A	49.3±1.7y	N/A	28.8±0.8(25-35)	Hypercholesterolemia	Total n=20	6wks	Walnuts and walnut oil	37g walnuts & 15g walnuts oil	AAD	100	Good	5

Wien et al., 2010 USA	RCT, parallel	Caucasian:38.5; Hispanic: 13.8; African American : 35.4; Asian:12.3	53.5±10y	26.2	29.5±5	Prediabetes	Almonds n=32; Control n=33	16wks	Almonds diet	60g/d	No nuts	83.1	Good	5
Wu et al., 2014 USA	RCT, crossover	Caucasian	60±SEM 1y	N/A	24.9±0.6	Healthy (post-menopausal)	Walnut n=24; Control n=28	8wks	Walnut diet	43g/d	Western-type diet	70.2	Good	5
Zibaenezhad et al., 2017 Iran	RCT, double-blinded, parallel	N/A	54.8±11.05y(35-75y)	58.9	27.4±2.35	Hyperlipidemia & T2DM	Walnut n=48; Placebo n=48	90d(12.9wks)	Walnut oil with foods	15g/d	Distilled water	93.8	Good	5

Table 3.1.1 RCTs eligible for inclusion in the systematic review and meta-analysis in East Asians.

(Author/ year) country of origin	Study design	Ethnicity (%)	Mean ± SD/SEM or range Age	Sex %male	Mean BMI (kg/ m ²)	Health y status	Baselin e sample size	Intervention length	Intervention	Dose & frequen cy	Control/co mparator	Retention rate
Hiraoka- Yamamoto et al., 2004 Japan	RCT, parallel	Japanese	19.5±0.1y(18- 24y)	0	20.45±SEM0. 4	Healthy	Coconut n=24; Macadamia n=24	3wks	Macadamia nuts bread	10g/d	Coconuts breads	100
Iwamoto et al., 2002 Japan	RCT, crossover, single- blinded	Japanese	23.7±SEM0.9y	50	21.45±SEM0. 5	Healthy	Total n=40	4wks	Walnut diet	51g/d	Reference diet	100
Jung, H et al.,2018 Korean	RCT, crossover	Korean	52.4±0.6y	13.1	25.4±0.22	Overweight or obese	Almond n=45; Cookie n=45	4wks	Roas ted almo nds	56g/d	70 g/d isocaloric home- made cookies	93
Lee et al., 2014 South Korean	RCT, parallel	Korean	35-65y	0	27.1±2.1	MS	Nut n=30; Control n=31	6wks	Mixed nuts (walnuts, peanuts, and pine nuts)	30g/d	Control diet	98.36
Li et al., 2011 Taiwan China	RCT, crossover	Chinese	58±SEM2y	45	26.0±SEM0.7	T2DM	Total n=22	4wks	Roasted, unsalted whole almonds diet	56g/d	Program step II diet	90.90

Liu et al., 2013 Taiwan China	RCT, crossover	Chinese	58±SEM2y(40-70 y)	N/A	26.0±SEM0.7	T2DM with mild hyperlipidemia	Total n=22	4wks	Almond diet	56g/d	NCEP step II diet	90.9
Wu et al., 2010 Shanghai China	RCT, parallel	Chinese	48.4±8.2y	56.4	25.4±2.6	MS	LCF n=94; LCW n=94	12wks	Walnuts with lifestyle counseling (LCW)	30g/d	Flaxseed with LCW	100

Table 3.2. Pooled estimates of positive effect size for the results of nuts interventions compared to respective controls.

Outcome Parameter	Mean Difference	95% Confidence Interval	p-Value	No. of Studies	Sample Size	I² (%)
FFA (mmol/L)	-0.03	-0.05 to -0.01	<i>P</i> =0.0009	4	156	0
TC (mg/dl)	-7.23	-9.66 to -4.81	<i>P</i> < 0.001	72	3718	59
HDL-C (mg/dl)	0.9	0.14 to 1.67	<i>P</i> =0.02	73	3760	53
LDL-C (mg/dl)	-6.81	-8.81 to -4.8	<i>P</i> < 0.001	74	3756	68
TG (mg/dl)	-8.66	-12.58 to -4.74	<i>P</i> < 0.001	71	3725	64
VLDL-C (mg/dl)	-1.85	-2.96 to -0.74	<i>P</i> =0.001	15	1127	0
Apo B (mg/dl)	-4.60	-6.87 to -2.34	<i>P</i> < 0.001	27	1299	64

Table 3.3 The dose of nuts in this systematic review and meta-analysis

	Walnuts	Almonds	Pistachios	Brazil nuts	Peanuts	Macadamia	Hazelnuts	Cashews	Pecans	Mixed nuts
Number of papers	26	20	12	3	4	4	5	4	3	7
Average dose (per day)	48g	54.2g	75g	25.3g	66.5 g	26.25g	45.7g	61.8g	60.8g	45.8g
Dose range (per day)	15 – 85.5g	10 – 100g	49-94.5g	13-50g	56-77g	10-42.5g	29-67.5g	30-85.5g	42.5-72g	30-80g
No. dose	N=1; Walnuts (Juglans regia L.)	N=1; 15% total energy from almonds	N=1; 20% total energy from pistachios	None	N=2; 20kcal/kg; N/A	N=2; N/A; 50% total fat	None	None	None	N=1; 7 kCal /kg bodyweight daily;

***Argan oil: Argan oil (n=1), which is derived from the nut of the argan tree is 25ml/d.**

Table 3.4. Subgroup analysis was undertaken based on the participants' country as well as ethnicity. East Asian and Non-East Asian population in both ethnicity and country were analysed for nuts consumption.

Outcome parameter	Subgroup	Subgroup division	Mean Difference; 95% Confidence Interval;	No. of Studies	P-value & I² (%) in subgroups	p-Value and I² (%) of subgroup differences
TC	Ethnicity	East Asian	-4.74 (95% CI: -10.90 to 1.43)	6	P=0.13; I ² =0%	P=0.48; I ² =0%
		Non-East Asian	-7.91 (95% CI: -13.69 to -2.12)	18	P=0.002; I ² =64%	
	Country	East Asian	-4.74 (95% CI: -10.90 to 1.43)	6	P=0.13; I ² =0%	P=0.42; I ² =0%
		Non-East Asian	-7.50 (95% CI: -10.10 to -4.91)	67	P<0.00001; I ² =59%	
HDL-C	Ethnicity	East Asian	-0.08 (95% CI: -2.29 to 2.14)	6	P=0.94; I ² =0%	P=0.29; I ² =10.2%
		Non-East Asian	1.26 (95% CI: 0.44 to 2.08)	18	P=0.007; I ² =7%	
	Country	East Asian	-0.08 (95% CI: -2.29 to 2.14)	6	P=0.94; I ² =0%	P=0.38; I ² =0%
		Non-East Asian	0.98 (95% CI: 0.17 to 1.79)	67	P=0.02; I ² =55%	
LDL-C	Ethnicity	East Asian	-4.77 (95% CI: -10.67 to 1.12)	6	P=0.06; I ² =0%	P=0.79; I ² =0%
		Non-East Asian	-5.97 (95% CI: -9.83 to -2.12)	18	P=0.0004; I ² =44%	
	Country	East Asian	-4.78 (95% CI: -9.85 to 0.30)	6	P=0.06; I ² =0%	P=0.43; I ² =0%
		Non-East Asian	-7.0 (95% CI: -9.13 to -4.88)	68	P<0.00001; I ² =69%	
TG	Ethnicity	East Asian	-1.16 (95% CI: -9.39 to 7.07)	6	P=0.56; I ² =0%	P=0.21; I ² =36.4%
		Non-East Asian	-9.46 (95% CI: -20.54 to 1.61)	18	P=0.04; I ² =78%	
	Country	East Asian	-2.10 (95% CI: -9.35 to 5.16)	6	P=0.57; I ² =0%	P=0.09; I ² =64.9%
		Non-East Asian	-9.33 (95% CI: -13.56 to -5.10)	66	P<0.00001; I ² =63%	
Oxidized-LDL	Ethnicity	East Asian	-6.65 (95% CI: -29.44 to 16.14)	2	P=0.57; I ² =81%	P=0.73; I ² =0%
		Non-East Asian	-1.69 (95% CI: -18.34 to 14.96)	1	P=0.84	
	Country	East Asian	-6.65 (95% CI: -29.44 to 16.14)	2	P=0.57; I ² =0%	P=0.58; I ² =0%
		Non-East Asian	-0.17 (95% CI: -0.55 to 0.22)	9	P=0.39; I ² =81%	
SBP	Ethnicity	East Asian	-4.21 (95% CI: -9.09 to 0.67)	2	P=0.09; I ² =0%	P=0.25; I ² =23.7%
		Non-East Asian	-1.69 (95% CI: -3.52 to 0.14)	6	P=0.26; I ² =0%	
	Country	East Asian	-0.67 (95% CI: -4.57 to 3.23)	4	P=0.74; I ² =30%	P=0.36; I ² =0%

		Non-East Asian	1.58 (95% CI:-1.28 to 4.43)	37	P=0.28; I ² =90%	
DBP	Ethnicity	East Asian	-2.2 (95% CI:-9.41 to 5.01)	1	P=0.55	P=0.58; I ² =0%
		Non-East Asian	-0.04 (95% CI: -2.77 to 2.69)	6	P=0.93; I ² =53%	
	Country	East Asian	-0.67 (95% CI:-4.57 to 3.23)	4	P=0.74; I ² =30%	P=0.36; I ² =0%
		Non-East Asian	1.58 (95% CI:-1.28 to 4.43)	34	P=0.28; I ² =90%	
FFA	Ethnicity	East Asian	0.00 (95% CI:-0.16 to 0.16)	1	P=1	P=0.67; I ² =0%
		Non-East Asian	-0.03 (95% CI:-0.06 to -0.01)	2	P=0.0008; I ² =0%	
	Country	East Asian	0.00 (95% CI:-0.16 to 0.16)	1	P=1	P=0.67; I ² =0%
		Non-East Asian	-0.03 (95% CI:-0.06 to -0.01)	3	P=0.0008; I ² =0%	
Adiponectin	Ethnicity	East Asian	0.02 (95% CI:-4.28 to 4.32)	2	P=0.97; I ² =0%	P=0.25; I ² =25.6%
		Non-East Asian	0.78 (95% CI:-0.1 to 1.46)	3	P=0.02; I ² =31%	
	Country	East Asian	-0.03 (95% CI:-1.27 to 1.21)	1	P=0.96	P=0.15; I ² =51.7%
		Non-East Asian	0.92 (95% CI: 0.54 to 1.3)	7	P<0.00001; I ² =0%	
Lag time of LDL	Ethnicity	East Asian	13 (95% CI: 8.57 to 17.43)	1	P<0.00001	Not applicable
		Non-East Asian	Not applicable	0	Not applicable	
	Country	East Asian	13 (95% CI: 8.57 to 17.43)	1	P<0.00001	P=0.0004; I ² =92%
		Non-East Asian	-8.4 (95% CI:-19.42 to 2.62)	1	P=0.14	
TAC	Ethnicity	East Asian	0.06 (95% CI:-0.19 to 0.31)	1	P=0.64	Not applicable
		Non-East Asian	Not applicable	0	Not applicable	
	Country	East Asian	0.06 (95% CI:-0.19 to 0.31)	1	P=0.64	P<0.0001; I ² =94.8%
		Non-East Asian	2.31 (95% CI: (1.34 to 3.29)	2	P<0.00001; I ² =0%	
Apo A	Ethnicity	East Asian	0.00 (95% CI:-29.97 to 29.97)	2	P=0.56; I ² =0%	P=0.70; I ² =0%
		Non-East Asian	-0.85 (95% CI:- 4.89 to 3.19)	5	P=0.68; I ² =0%	
	Country	East Asian	-1.4 (95% CI:-8.3 to 5.49)	3	P=0.69; I ² =0%	P=0.84; I ² =0%
		Non-East Asian	-0.64 (95% CI: -3.43 to 2.14)	23	P=0.65; I ² =39%	
Apo B	Ethnicity	East Asian	-11.81 (95% CI: -29.66 to 6.04)	2	P=0.19; I ² =70%	P=0.53; I ² =0%
		Non-East Asian	-6.05 (95% CI: -8.48 to -3.62)	7	P<0.00001; I ² =0%	
	Country	East Asian	-5.91 (95% CI: -16.06 to 4.24)	3	P=0.25; I ² =65%	P=0.81; I ² =0%

		Non-East Asian	-4.61 (95% CI: -6.98 to -2.24)	25	P=0.0001; I ² =66%	
Hs-CRP	Ethnicity	East Asian	-0.10 (95% CI: -0.35 to 0.15)	1	P=0.43	P=0.71; I ² =0%
		Non-East Asian	-0.24 (95% CI: -0.94 to 0.46)	3	P=0.50; I ² =14%	
	Country	East Asian	-0.1 (95% CI: -0.35 to 0.15)	1	P=0.43	P=0.19; I ² =40.9%
		Non-East Asian	-0.36 (95% CI: -0.66 to -0.06)	10	P=0.02; I ² =0%	
CRP	Ethnicity	East Asian	-1.29 (95% CI: -2.98 to 0.4)	1	P=0.14	P=0.23; I ² =31.8%
		Non-East Asian	-0.21 (95% CI: -0.64 to 0.22)	3	P=0.34; I ² =0%	
	Country	East Asian	-1.29 (95% CI: -2.98 to 0.4)	1	P=0.14	P=0.14; I ² =53.6%
		Non-East Asian	-0.02 (95% CI: -0.08 to 0.04)	15	P=0.48; I ² =0%	
TNF-a	Ethnicity	East Asian	-0.06 (95% CI: -0.13 to 0.01)	1	P=0.1	P=0.4; I ² =0%
		Non-East Asian	-1.07 (95% CI: -3.41 to 1.28)	3	P=0.37; I ² =60%	
	Country	East Asian	-0.06 (95% CI: -0.13 to 0.01)	1	P=0.1	P=0.77; I ² =33%
		Non-East Asian	0.03 (95% CI: -0.25 to 0.31)	8	P=0.85; I ² =39%	
IL-6	Ethnicity	East Asian	0.29 (95% CI: -0.46 to 1.04)	2	P=0.45; I ² =75%	P=0.33; I ² =0%
		Non-East Asian	-0.11 (95% CI: -0.37 to 0.16)	2	P=0.69; I ² =48%	
	Country	East Asian	0.29 (95% CI: -0.46 to 1.04)	2	P=0.45; I ² =75%	P=0.46; I ² =0%
		Non-East Asian	0 (95% CI: -0.09 to 0.1)	11	P=0.92; I ² =0%	
sICAM-1	Ethnicity	East Asian	0.10 (95% CI: -28.17 to 28.36)	2	P=0.99; I ² =0%	P=0.76; I ² =0%
		Non-East Asian	-3.24 (95% CI: -20.49 to 14.02)	3	P=0.71; I ² =0%	
	Country	East Asian	0.1 (95% CI: -28.17 to 28.36)	2	P=0.99; I ² =0%	P=0.74; I ² =0%
		Non-East Asian	-4.88 (95% CI: -12.55 to 2.79)	12	P=0.21; I ² =0%	
sVCAM-1	Ethnicity	East Asian	-48.61 (95% CI: -97.96 to 0.74)	2	P=0.05; I ² =0%	P=0.13; I ² =55.8%
		Non-East Asian	14.24 (95% CI: -51.12 to 79.6)	2	P=0.67; I ² =0%	
	Country	East Asian	-48.61 (95% CI: -97.96 to 0.74)	2	P=0.05; I ² =0%	P=0.08; I ² =66.9%
		Non-East Asian	5.65 (95% CI: -30.49 to 41.79)	10	P=0.76; I ² =0%	

Table 3.5. Study quality

References	Generation of Random method clarification	Method of monitoring subject compliance to nuts consumption or/and reference diets	Drop-outs reasons clarification/withdraw reasons	Allocation concealment of treatment
Agebratt et al., 2016 Canada	Not reported	Food diary record	No dropouts	Not reported
Aronis et al., 2012 Greece	Not reported	Not reported	No dropouts	Not reported
Bamberger, C et al., 2017, Germany	Randomization (blocking of 12; SAS proc factex)	An in-patient setting or a setting where all of the meals are provided	Disease; Medication; Personal reason; Protocol violation	Not reported
Bento et al., 2014 Brazil	Not reported	3-d dietary record	Pregnant; personal unforeseen circumstances	Not reported
Berryman et al., 2015 USA	Computer-generated randomization	Daily weigh-ins and food logs	Diet issues; Time restraints; Unrelated illness; Moved out of area; Pre-existing metabolic condition	Not reported
Brennan et al., 2010 USA	Not reported	Weight foods	Not reported	Yes: blinded statistician assigned diets
Burns-Whitmore et al., 2014 USA	Not reported	Daily diary record; Fatty acid composition of erythrocyte membranes assessment	Family or job pressures; Allergies unrelated to interventions	Not reported
Carvalho et al., 2015 Brazil	Computer generated random list restricted in blocking of participants and sequentially numbered labels were inserted in sealed containers with nut or placebo	Plasma selenium levels assessment	Pleural effusion due to a history of CAD	Not reported
Casas-Agustench et al., 2011 Spain	Not reported	Extra packages of nuts were given to the rest of family	Personal reasons	Not reported

Chen et al., 2015 USA	The permuted blocks of size 6 (total 10 blocks)	Phone calls; Package bags check	Colitis; Body weight	Not reported
Chiang et al., 2012 USA	Not reported	Direct observation during meal times; Dietary diaries check	Not reported	Not reported
Chisholm et al., 1998 New Zealand	Not reported	The relative increases of linoleic acid in plasma triacylglycerol and alpha linolenic acid assessment; Dietary record check	No dietary records provide	Not reported
Chisholm et al., 2005 New Zealand	Not reported	Not reported	Not reported	Not reported
Claesson et al., 2009 Sweden	Not reported	Not reported	Gastrointestinal symptoms; Diarrhoea and increased flatus	Not reported
Colpo et al., 2014 Brazil	Latin squares of 4*4	24-h dietary recall; Food frequency questionnaires	No dropouts	Not reported
Curb et al., 2000 USA	Randomizations stratified by sex	Telephone screening; Individual and group meetings	Not reported	Yes: study personnel involved in performing measurements and analyses were blinded to the diet sequences.
Damavandi et al., 2013 Iran	Not reported	Not reported	Not reported	Not reported
Davidi et al., 2011 USA	Random number generated by SAS	Empty packages collection; A log detailing the compliance to fill out	Did not come for end of visit; Discontinued intervention due to high LDL, high HDL levels and was misplaced to the wrong treatment group	Not reported
de Souza et al., 2018 Brazil	Random numbers allocated consecutively to the subjects in the order that they attended the randomization visit	Telephone calls and during routine monthly consultations	Pregnancy; Change of address; started a physical exercise program; missed evaluations due to work commitment or illness among family members	Not reported

Dhillon et al., 2016 USA	Not reported	24-h food recalls; Weight check	Illness; Pregnancy; Time constraints	Not reported
Din et al., 2011 UK	Block randomization	Food diary record	No dropouts	Not reported
Foster et al., 2012 USA	Random number generator	Not reported	Time constraints/work schedule; Dissatisfied with program; Life stressors; No reason given; Pregnancy; Relocation	Not reported
Gebauer et al., 2008 USA	Not reported	Dietary questionnaires	No dropouts	Yes: study personnel who measured outcome variables were blinded to the diet assignments
Ghadimi Nouran et al., 2010 USA	Not reported	An extra coded bag of peanuts to share with family and friends; 24h diet recalls	Lost interest; Unforeseen travel	Not reported
Griel et al., 2008 Canada	Not reported	The review of daily and weekly monitoring forms	Time constraints	Not reported
Gulati et al., 2014 India	Not reported	Weekly compliance questionnaires, telephone calls, discussion, and crosschecking with the spouse or any close relative; Empty packets of pistachios and pistachio shells; Food-frequency questionnaire and 24-h dietary recall	Not reported	Not reported
Hiraoka-Yamamoto et al., 2004 Japanese	Not reported	Calculation of percentages of the prescribed bread consumed	No dropouts	Not reported
Huguenin et al., 2015a Brazil	The randomization was in blocks of 10 and based on a table of random numbers	Not reported	Personal reasons; Pulmonary edema; Living far; Financial reason; Requiring accompaniment; Inpatient status; Cancer diagnosis	Yes: the randomization were blinded except for one who encoded interventions and had no contact with the center

				at which the study was conducted
Iwamoto et al., 2002 Japan	Not reported	Tray checks at the meals eaten on site and by self-report on standardized forms for the packed meals	No dropouts	Not reported
Jamshed et al., 2015 Pakistan	Computer-generated block randomization	Regular phone calls; Dietary diary record	Fail to contact; Started using nuts; Left the city; Angioplasty; Discontinued	Not reported
Jenkins, et al., 2018 Canada	Randomisation was carried out anonymously	7-day food record	Allergy	Not reported
Jenkins et al., 2002 USA	Not reported	7-d diet record, a supplement checklist on which subjects recorded supplements consumed and return of uneaten supplements, which were weighed and recorded	Reasons directly related to the study: food allergies and abdominal discomfort; unrelated reasons	Not reported
Jung, H et al., 2018 Korean	Not reported	A diary calendar and counting returned packages; 3-day dietary records	Time commitment; Abdominal discomfort	Not reported
Kasliwal et al., 2015 India	Not reported	Monthly visits	Participants core laboratory results were outside the study eligibility criteria	Not reported
Katz et al., 2012 USA	Not reported	3-day diet record	Changes in medication; Inability to comply with the protocol; Schedule conflicts	Not reported
Kay et al., 2010 UK	Not reported	Food questionnaires	Not reported	Not reported
Kendall et al., 2014 Canada	Not reported	Not reported	No dropouts	Not reported
Kocyigit et al., 2006 Turkey	Not reported	Not reported	No dropouts	Not reported

Kris-Etherton et al., 1999 USA	Random, balanced order sequence	Body weight measurements; Daily dietary questionnaire	No enthusiasm about the length of the study; Different geographical areas	Not reported
Kurlandsky and Stote, 2006 USA	Not reported	3-day diet record	Minor illness and a total cholesterol level below inclusion standards	Not reported
Lee et al., 2014 South Korean	Not reported	Daily dietary self-record	Personal reasons	Not reported
Lee et al., 2017 USA	Computer-generated randomization scheme utilized a Williams design with permuted block randomization	Daily weight logs and daily food logs checks	Not compliant; Food dislikes; Time restraints; Relocation; Personal reasons	Not reported
Li et al., 2011 Taiwan China	1:1 manner using a computerized random proportion model	Daily diet diary to record foods not eaten, non-study foods eaten, and beverages	Noncompliance in consuming the study meals	Not reported
Li et al., 2010 USA	Not reported	Meet with research dietitians	Noncompliant with the daily food log or study visit or lost to follow-up and consequently drop from the study; Rash	Not reported
Liu et al., 2013 Taiwan China	Not reported	Daily diet diary to record foods not eaten, non-study foods eaten, and beverages	Without full compliance	Not reported
Lopez-Uriarte et al., 2010 Spain	Not reported	3-day food record	Personal reasons	Not reported
Ma et al., 2010 USA	Two possible sequence permutations	3-day diet record	Changes in medications; Poor compliance with the treatment protocol	Not reported
Mah et al., 2017 USA	Statistician generates the randomization for the intervention sequence with the use of the SAS PROC PLAN with a 1:1 allocation ratio	Returned food items	Adverse events; An unwillingness to comply with the study diet	Not reported

McKay, et al., 2018 USA	Randomization was stratified by gender according to a computer-generated list	Empty containers and unused food return	Elevated TC, LDL, SBP,DBP, fasting blood glucose levels; low HDL, obese	Study personnel were blinded to the treatment assignment for the duration of the intervention and sample analysis
Mohamedou et al., 2012 Morocco	Not reported	Not reported	Personal reasons	Not reported
Mohan et al., 2018 India	Not reported	Self-reported dietary intake by 24-h dietary recall; face-to-face interview	Allergy; Personal reasons; Non-responsive	Not reported
Moreira Alves et al., 2014 Brazil	Not reported	Time records in a notepad daily; 3-day food record	Not reported	Not reported
Morgan et al., 2002 USA	Not reported	3-d dietary record	Withdrew consent; Intolerance to the walnuts	Not reported
Morgan and Clayshulte, 2000 USA	Not reported	3-day food record; Food frequency checklists; Interviewing participants at each study visit and inspecting food ration boxes	Unable to confirm the study protocols	Not reported
Mukuddem-Petersen et al., 2007 UK	Randomly drawing numbers by a hat	A dietitian supervised mealtimes and ensured the complete intake of foods; Food diaries of the additional points used and possible left-overs were collected and weighed	Work obligations; Unrelated medical condition; Holiday	Not reported
Nieman et al., 2014 USA	Randomized (1:1 allocation, random number generator)	Email check weekly; Empty plastic bags check	Not reported	Not reported
Njike et al., 2015 USA	SAS-generated random table (a permuted design in a 1:1 ratio)	Not reported	Medical reasons unrelated to walnut consumption; Relocation; Allergic reaction; Inability to comply with the study	Not reported

			protocol; Mental and family issues	
Olmedilla-Alonso et al., 2008 Spain	Not reported	Dietary record	No dropouts	Not reported
Orem et al., 2013 Turkey	Not reported	Not reported	No dropouts	Not reported
Parham et al., 2014 Iran	The blocks using random numbers	Not reported	Poor compliance with the study protocol	Not reported
Perez-Martinez et al., 2007 Spain	Not reported	Not reported	No dropouts	Not reported
Rajaram et al., 2001 USA	Randomly assigned after stratification based on two categories of age, gender and screening values of serum cholesterol	Food diary record; Plasma fatty acids assessment	No dropouts	Not reported
Rajaram et al., 2010 USA	Random, balanced order sequence	Interventions were eaten under the supervision of a senior investigator	Lack of adherence to the study protocol	Not reported
Rajaram et al., 2009 USA	Randomized and stratified on the basis of age, gender, and baseline serum TC concentration to 1 of 6 possible diet sequences	Foods weight; Daily diary record	Time conflicts	Not reported
Ros et al., 2004 Spain	Not reported	7-day dietary recalls	Personal reasons	Not reported
Ruisinger et al., 2015 USA	Not reported	3-day food record	The inability to consume foods daily and concerns about the additional energy intake	Not reported

Sabate et al., 1993 USA	Stratification on the basis of age, baseline serum cholesterol level and body mass index	Food diaries record	Blood drawing missing	Not reported
Sabate et al., 2003 USA	Not reported	Not reported	Unable to comply with the diet protocol	Not reported
Sauder et al., 2014 USA	Not reported	Daily food compliance questionnaires; Returned food containers check	Developed food intolerance; Pre-existing medical condition revealed	Not reported
Sauder et al., 2015 USA	Simple randomization	Not reported	Elevated blood pressure; Medication change; No longer interested; Developed food intolerance; Pre-existing medical condition revealed	Not reported
Schutte et al., 2006 South Africa	Stratified based on gender and age	Weighing returned leftover food portions and checking food diaries	No dropouts	Not reported
Sheridan et al., 2007 USA	Not reported	Pistachio storage bags return	No dropouts	Not reported
Sola et al., 2012 Spain	Computer-generated random number sequence in gender-stratified blocks.	Empty wrapper counts and any non-consumed doses check	Low compliance rate (<80%) of interventional consumption	Yes: clinical investigators and laboratory personnel were blinded with respect to the type of cream being consumed.
Somerset et al., 2013 Australia	Randomised consecutively as subjects entered the trial, in blocks of 10 subjects	Not reported	No dropouts	Not reported
Spiller et al., 1998 Italy	Not reported	3-day diet record; 24-hour dietary recalls; Verbal reports at the study group meetings	Reasons unrelated to the study; Unwilling to follow a diet expected not to lower TC	Not reported
Sweazea et al., 2014 USA	Not reported	Not reported	Scheduling conflicts	Not reported

Tamizifar B, 2005 Iran	Not reported	Food diaries record	Pre-existing gastrointestinal problems	Not reported
Tapsell et al., 2009 Australia	Computerized random number generator	Biomarker data analysis	Work/family time commitments; Indigestion problems; Consulted naturopath and started fish oil capsules; Travelling; Moved out of area	Not reported
Tapsell et al., 2004 Australia	Not reported	Not reported	Not reported	Not reported
Tey et al., 2011 New Zealand	Blocks of size four to allocate and incomplete blocks remaining at the conclusion of enrolment were randomly allocated first using strata based on sex and BMI	Weighing returned serving bags; 3 day dietary record	Dissatisfaction with group assignment; Personal issues; Adverse events	Not reported
Tey et al., 2013 Australia	Not reported	Weighing bags returned; 3 day dietary record	BMI<25; Pregnant	Not reported
Torabian et al., 2010 USA	Not reported	24h dietary recalls	Diagnosed with a metabolic disorder; Lipid-lowering medications usage	Not reported
West et al., 2012 USA	Not reported	Not reported	No dropouts	Not reported
West et al., 2010 Canada	Randomization table to a counterbalanced sequence of diets	Not reported	No dropouts	Not reported
Wien et al., 2010 USA	Randomized without stratification using computer-generated random integer generator software (www. random.org)	Plasma atocopherol concentrations evaluation; Self-reported food dietary record	Work and personal schedule conflicts	Not reported
Wu et al., 2010 Shanghai China	Block randomized to 1 of the 3 intervention arms	The weight of breads consumed divided by the prescribed weight of total breads throughout the intervention; ALA content of erythrocyte membranes assessment	No dropouts	Not reported

Wu et al., 2014 USA	Complete block design	Not reported	Protocol violations; Acute infection with the use of antibiotics>5 days; Cortisone injections; Dietary non-compliance; Abnormal thyroid function after subtotal thyroidectomy	Not reported
Zibaeenezhad et al., 2017 Iran	Computer-based random digit generator based on the registration number of participants	Not reported	Fail to blinding	Not reported

CHAPTER 4

Effects of olive oil on cardiovascular risk factors in different ethnic groups: A systematic review and meta-analysis of randomized controlled trials

Abstract

Background and aims. Epidemiological evidence suggests an association between consumption of olive oil and lower risk for cardiovascular diseases (CVDs). However, how factors such as ethnicity plays a role in this association are not well established. Therefore, the aim of the present systematic review and meta-analysis was to synthesize data from randomized controlled trials (RCTs) investigating the effects of olive oil on markers of cardiovascular biomarkers.

Methods. Literature search in electronic databases including Medline, Web of Science and Scopus were searched from inception to December 2018. Inclusion criteria were: intervention RCTs reporting effects of olive oil on CV risk factors among adults. The main outcomes of interest included blood lipids (total-, HDL-C, LDL-C, TG, VLDL-C, Apo A-I, Apo B, ox-LDL), endothelial function (FMD, PWV, NO), SBP, DBP and inflammatory factors (CRP, hs-CRP, IL-6, IL-8, IL-10, sICAM-1, sVCAM-1, TNF-alpha, adiponectin, E-selectin) and other biomarkers (VWf; fibrinogen and ET-1). Random-effects models were used to determine the pooled effect sizes. Systematic review registration: **CRD42018089055**.

Results. Out of 791 publications identified, 22 randomized controlled trials were included in the final selection and were meta-analyzed. Overall, olive oil (with daily consumption ranging between 13.3 and 64.8 gram) improve markers such as PAI-1 and tPA although tPA were not included into the meta-analysis as the significant result of tPA was only from single study.

Limitations. The small number of studies/participants limits this review.

Conclusions. The available evidence on the effects of olive oil on CV risk factors supports the view that variables PAI-1, tPA are shown to be improved after olive oil consumption. More studies from Asian and non-Asian countries regarding olive oil intake and cardiovascular risk factors need to be researched in the near future.

4.1 Introduction

Consumption of olive oil has almost doubled over the last 25 years with an increase of 73% as the growth in global demand for olive oil has been powered in part by the health benefits, especially for cardiovascular health. Olive oil has the biggest growth (41%) among all products exports to China from Italy (Ylenia 2016). China has imported approximately 12.25 million tons of olive oil in 2014 while in Britain, olive oil market is estimated around £250M (CBI - Ministry of Foreign Affairs 2020). The UK's imports of olive oil exceeded 84 thousand tones, at a value of €220 million in 2019.

The popularity of olive oil consumption is because olive oil contains a complex mixture of over 200 compounds which are beneficial for health (Aiello, Guccione et al. 2015). The main constituents of olive oil are triglycerides (98-99%) and the three main fatty acids in the triglyceride fraction are a monounsaturated fatty acid (MUFA) (oleic acid), a saturated fatty acid (palmitic acid) and a polyunsaturated fatty acid (PUFA) (linoleic acid) (Aiello, Guccione et al. 2015). Oleic acid represents the topmost MUFA provided in the diet (~90% of all MUFAs) and is the main MUFA of olive oil (55-83%). Oleic acid was shown to enhance the resistance of LDL-C to oxidation and therefore, reduces the risk of atherosclerosis (Aiello, Guccione et al. 2015). The remaining unsaponifiable fraction (1-2%) contains phenolic compounds and the phenolic fraction in olive oil are polyphenols of which there are 7 different subfamilies. These are anthocyanins, flavonoids, flavones, phenolic acids, phenolic alcohols, acids and secoiridoids. Their amount in olive oil is highly affected by the variety as well as the geographical origin of the olives (Aiello, Guccione et al. 2015). Phenolic compounds have antioxidant, anti-inflammatory and antimicrobial properties, which reduce atherosclerotic plaque formation (Cicerale, Lucas et al. 2012, Virruso, Accardi et al. 2014).

Epidemiological research from the Mediterranean basin has suggested a lower incidence of coronary heart disease in people from Mediterranean countries where olive oil is the primary source of fat (Dontas, Zerefos et al. 2007). Epidemiological research consistently showed that the health benefit of olive oil is associated with increased longevity (Buckland and Gonzalez 2015) and this benefit is mostly due to the olive oil's unequivocal cardio-protective role (Guasch-Ferré, Hu et al. 2014). Beside the fact that MUFA and PUFAs benefit blood lipids (Puiggros, Chacon et al. 2002, Chan, Demonty et al. 2007), phenolic content in olive oil was also shown to reduce oxidative status, lowers blood pressure (Covas 2007) and preventing CVD risk factors, including diabetes, metabolic syndrome and obesity (Buckland and Gonzalez 2015).

Despite considerable evidence on the biological mechanisms involved (Covas, Konstantinidou et al. 2009, Estruch 2010, Lopez-Miranda, Perez-Jimenez et al. 2010), only up until have epidemiological studies provided direct evidence on the relationship between olive oil consumption and primary prevention of CVD (Ruiz-Canela and Martinez-Gonzalez 2011, Ros 2012). However, the EPIC-Spain cohort study observed a negative association between olive oil consumption and coronary heart disease (CHD) (Buckland, Travier et al. 2012). A three-city large-scale study presented results that intense olive oil consumption for dressing or cooking (37% of the cohort) had a 41(95% CI 6, 63) % reduced risk of stroke compared with participants who never consume olive oil (23% of the cohort) (Samieri, Feart et al. 2011). Additionally, a systematic review showed that incorporating with the Mediterranean diet olive oil intake might have a beneficial effect on endothelial function and markers of inflammation (Schwingshackl, Christoph et al. 2015). Most recently, new research (Xu, Wang et al. 2018) has revealed that unsaturated fats in olive oil promote a high level of Apo lipoprotein A-IV which is a protein that inhibits the aggregation of platelets, components of the blood that can clump together and form clots within arteries.

A number of studies supporting the health benefits of olive oil have been carried out among Mediterranean populations, such as the PREDIMED study. However, whether studies have evaluated the effects of olive oil among difference ethnicity groups is uncertain. Here we undertake a systematic review to explore whether studies comparing the effect of olive oil supplementation among different ethnic groups are available, with a particular interest on East Asian and Caucasians individuals. CV markers such as blood lipids (TC, HDL-C, LDL-C, TG ox-LDL), markers of endothelial dysfunction (NO, FMD, PWV, PWA), blood markers - cell adhesion molecules: VCAM-1, ICAM-1, E-selectin, vWf), oxidative damage (ox-LDL) and markers of inflammation (IL-6, IL-1 β , CRP, SAA) would also be targeted in this systematic review.

4.2 Materials and Methods

This systematic review was performed based on the established methods recommended by the Cochrane (Higgins, Altman et al. 2011) and the Centre for Reviews and Dissemination guidelines (Tacconelli 2010) (Centre for Reviews and Dissemination 2009). This systematic review is also reported with accordance to **PRISMA** criteria guidelines (Moher, Liberati et al. 2009) (**Appendix C1**). In addition, the protocol of the systematic review has been registered with PROSPERO, the International Prospective Register of Systematic Reviews (Centre For Reviews and Dissemination 2009), indexed under **Registration number CRD42018089055**.

Two researchers (FL and JL) documented the procedures to search and synthesize the evidence in accordance with the preferred reporting items for systematic review and meta-analysis (**PRISMA**) statement for reporting systematic reviews (**Figure. 4.1 and Table 4.1**) (Liberati, Altman et al. 2009, Higgins, Altman et al. 2011). FL and JL examined and selected the studies hierarchically. The decision was made initially on the basis of screening titles and abstracts of papers, and later the full text of the study was subsequently assessed to reach a decision.

4.2.1 Data sources and literature search strategy

Relevant studies were systematically identified by searching three electronic databases, including **MEDLINE** (beginning 1990); **Scopus** (beginning 1966); **Web of Science** (beginning 1991) through December 2018, with no language restriction.

The search strategies included the following terms: (“Cardiovascular risk factors” OR “Cardiovascular diseases”) AND (“endothelial function” OR “endothelial dysfunction” OR “flow-mediated dilation” OR “FMD” OR “arterial stiffness” OR “carotid intima-media thickness” OR “intercellular adhesion molecule-1” OR “ICAM-1” OR “vascular cell adhesion molecule-1” OR “VCAM-1” OR “e-selectin” OR “p-selectin” OR “dimethylarginine” OR “ADMA” OR “oxidized low density lipoprotein” OR “oxidized-LDL” OR “inflammation” OR “C-reactive protein” OR “CRP” OR “LDL cholesterol” OR “HDL cholesterol” OR “triglycerides” OR “total cholesterol”) AND (“olive oil” OR “EGCG”) AND (“randomized controlled trial” OR “randomized” OR “clinical trial as topic” OR “placebo” OR “randomly” OR “trial”) NOT (“animal”). Two investigators reviewed potentially relevant articles independently with discrepancies being resolved through consensus (FL). Institutional review board approval was not necessary for this systematic review.

4.2.2 Study selection

The following inclusion criteria were defined prior to study selection process:

- a) RCTs with either crossover or parallel design;
- b) Nutritional interventions: Interventions supplementing olive oil versus a control or placebo group (inactive);
- c) Adults participants more than 18 years of age;
- d) Minimum intervention duration was more than one week;

- e) Assessment of the “outcome of interest”: RCTs had to assess at least one of these primary outcomes. **Markers of inflammation** (CRP, IL-6, IL-8, TNF- α , adiponectin); **endothelial function/endothelial dysfunction** (sICAM-1, Svcam-1, FMD, PWV, NO); **Blood lipids** (plasma TC, HDL-C, LDL-C, VLDL-C, TG, Apo B and Apo A-I); **Fibrinolytic variables** (tPA, PAI-1); Vwf; Fibrinogen and ET-1.
- f) Report of post-intervention mean values (or if not available, change from baseline values were used instead) with standard deviation (or basic data which allow to calculate these parameters, i.e., standard errors, 95% confidence interval, p-values).

Exclusion criteria included:

- e) Non-randomized controlled trials;

Interventions not involving olive oil or combined interventions in which the effects of olive oil cannot be singled out;

4.2.3 Data extraction and statistical analysis

Data extracted from each trial were: study design, year of publication, country of origin, randomization, duration and length of follow-up, methods of analysis, completion rates; participant characteristics (population, settings of interventions, baseline characteristics); outcome measures (dietary and/or nutritional intake, BMI, CV biomarkers); intervention details (i.e. olive oil). Study quality was assessed using the Cochrane risk of bias tool (Higgins and Green 2011).

All data were analyzed using the software REVIEW MANAGER 5.3. A random effects model accounting for inter-study variation was used, thereby minimizing potential bias due to methodological differences between studies. In a random effects model, the post-mean values or the changes from baseline values and corresponding standard deviations of intervention and control/intervention groups were compared. Pooled results were reported as mean differences with 95% CIs and with two-sided P-values. Nevertheless, when variables (such as IL-6 or CRP, and ICAM-1) were reported on different scales, mean differences (MD) were used as a summary statistic for comparing effect sizes across studies.

Multiple dietary intervention arms from three studies were included in the meta-analysis. Following previous guidance (Higgins, Altman et al. 2011) excessive weightings from “double counts” arising from the “shared” group (in this case, the control group) were controlled by splitting the sample size of the shared group into approximately equal smaller groups for the comparisons. In this analysis, we sought to extract and analyse adjusted results from multivariate models, if reported in the studies.

Heterogeneity was evaluated using the I^2 statistic (Centre for Reviews and Dissemination 2009, Higgins, Altman et al. 2011) Levels of heterogeneity are commonly regarded as high when I^2 values are >50%. Publication bias was appraised by visually inspecting the funnel plot, and supplemented with calculations of the Egger's regression test (Egger, Davey Smith et al. 1997). Quality of studies was assessed using the jaded system (Jadad, Moore et al. 1996).

4.2.4 Quality assessment

The Cochrane collaboration's tool for assessing risk of bias was utilized to elucidate the risk of bias of the included studies attaching either low, unclear or high risk of bias to the seven domains (sequence generation, allocation concealment, blinding of participants and personnel, blinding of outcome assessment, incomplete outcome data, selective outcome reporting) to each study (Higgins, Altman et al. 2011, Higgins and Green 2011).

4.3. Results

4.3.1 Literature Search and Study Characteristics

Searches of the literature identified 791 published studies and the abstracts were reviewed. In total, 573 records remained after removing duplicates and 432 did not meet the inclusion criteria and were excluded. 141 studies were screened as full text and **Figure 4.1**. Ultimately, 22 studies were included in the present systematic review and meta-analysis (**Table 4.1**) (Sirtori, Gatti et al. 1992, Lichtenstein, Ausman et al. 1993, Kris-Etherton, Pearson et al. 1999, Pedersen, Baumstark et al. 2000, Nielsen, Pedersen et al. 2002, Perona, Canizares et al. 2004, Binkoski, Kris-Etherton et al. 2005, Cicero, D'Addato et al. 2009, Jiménez-Gómez, López-Miranda et al. 2009, Tholstrup, Hjerpsted et al. 2011, Kontogianni, Vlassopoulos et al. 2013, Namayandeh, Kaseb et al. 2013, Oliveras-Lopez, Molina et al. 2013, Engel and Tholstrup 2015, Maki, Lawless et al. 2015, Tong, Rappold et al. 2015, Venturini, Simao et al. 2015, Voon, Ng et al. 2015, de Oliveira, Kovacs et al. 2017, Atefi, Pishdad et al. 2018, Galvão Cândido, Xavier Valente et al. 2018, Khaw, Sharp et al. 2018).

Characteristics of the sample, interventions, outcome assessment and results are shown in **Table 4.1**. In addition, the study selection procedure is shown in **Figure 4.1**.

The 22 randomized controlled trials selected, comprised with altogether 860 participants who were followed-up for 5.3 weeks on average (range from 3 weeks to 13 weeks). Twelve out of 22 RCTs had a crossover design. The ages of the samples in these RCTs ranged from 18 to 78 years of age and the mean \pm SD of ages is 54.17 ± 6.82 . 4 studies recruited only male (Pedersen, Baumstark et al. 2000, Nielsen, Pedersen et al. 2002, Jiménez-Gómez, López-Miranda et al. 2009, Tholstrup, Hjerpsted et al. 2011) while 2 studies (Atefi, Pishdad et al. 2018, Galvão Cândido, Xavier Valente et al. 2018) recruited only female, the rest of studies involved mixed samples of men and women. Participants had existing disease conditions in 36% of all randomized trials; these were most commonly moderate hypercholesterolemia (n=5), Type 2 diabetic (n=1), obese or overweight (n=1), hypertensive (n=1) while 14 trials targeted at healthy participants (**Table 4.1**). Overall, 22 studies originated from the USA (n=7), Spain (n=3), Italy (n=2), Malaysia (n=1), Denmark (n=2), Brazil (n=3), Greece (n=1), UK (n=1) and Iran (n=2).

Eight studies (Cicero et al., 2009 , Galvão Cândido et al., 2018, Kontogianni et al., 2013, Khaw et al., 2018, Maki et al., 2015, Oliveras-Lopez et al., 2013, Pedersen et al., 2000, Venturini et al., 2015) which conducted trials by using extra virgin olive oil as interventions while the remaining studies used other olive oil such as virgin olive oil, regular olive oil and olive oil capsules. The dose of olive oil used in these interventions varied from 13.3 to 64.8 g/d and the mean dose is 36.71g/d in 14 studies. Two studies (Lichtenstein, Ausman et al. 1993, Binkoski, Kris-Etherton et al. 2005) reported the percentage of olive oil of the diet.

4.3.2 Main outcomes

Only variables including PAI-1 as well as t-PA out of 22 studies are shown to be improved after olive oil consumption (**Table 4.2**). 8 biomarkers including TC, HDL-C, DBP, sICAM-1, sVCAM-1, VWf, Apo B and IL-8 tend to be likely to be beneficial from olive oil interventions (**Appendix C2 – C22**). However, another 10 cardiovascular biomarkers including SBP, IL-6, CRP, adiponectin, TNF-alpha, Fibrinogen, Endothelin 1, plasma E-Selectin, ApoA1 and IL-10 show negative effects after olive oil intake (**Appendix C2 – C22**). LDL-C, TG and hs-CRP have no difference after olive oil interventions (**Appendix C2 – C22**).

4.3.2.1 Meta-analysis of studies supplementing olive oil

Olive oil interventions in two studies (Tholstrup, Hjerpsted et al. 2011, Tong, Rappold et al. 2015) having 58 participants resulted in a significant decrease in PAI-1 (MD: -1.02ng/mL, 95% CI: -1.92 to -0.12; $p = 0.03$, $I^2 = 0\%$) (**Figure 4.2**). Pooled estimates of effects size for all markers of inflammation are summarized in **Table 4.2**.

Overall, eight biomarkers were improved after olive oil consumption. Blood lipids such as TC (**Figure C2**), HDL-C (**Figure C3**), DBP (**Figure C7**), and inflammatory markers such as sICAM-1 (**Figure C8**), sVCAM-1 (**Figure C9**) and blood glycoprotein such as Vwf (**Figure C15**) and apo-lipoprotein such as Apo B (**Figure C19**) and chemokines such as IL-8 (**Figure C21**) are likely to slightly improve by olive oil consumption.

However, 10 biomarkers were more likely to present non-significant effect after olive oil consumption. These include SBP (**Figure C6**), pro-inflammatory cytokines such as IL-6 (**Figure C10**), CRP (**Figure C12**), adiponectin (**Figure C13**), TNF-alpha (**Figure C14**), glycoprotein such as fibrinogen (**Figure C16**) and endothelin-1 (**Figure C17**), and inflammatory biomarkers such as plasma E-selectin (**Figure C18**), apolipoprotein such as Apo A-I (**Figure C20**) and anti-inflammatory cytokines such as IL-10 (**Figure C22**). Hs-CRP (**Figure C11**), LDL-C (**Figure C4**) and TG (**Figure C5**) have no change after olive oil consumption.

4.3.2.2 Studies supplementing olive oil

Study (Tong, Rappold et al. 2015) reported that tissue plasminogen activator (tPA) levels were significantly increased immediately after (11.6%; 95% CI: 0.8, 22.2; $p = 0.04$) and 20 hours after concentrated ambient particulate exposure in the olive oil group (Tong, Rappold et al. 2015).

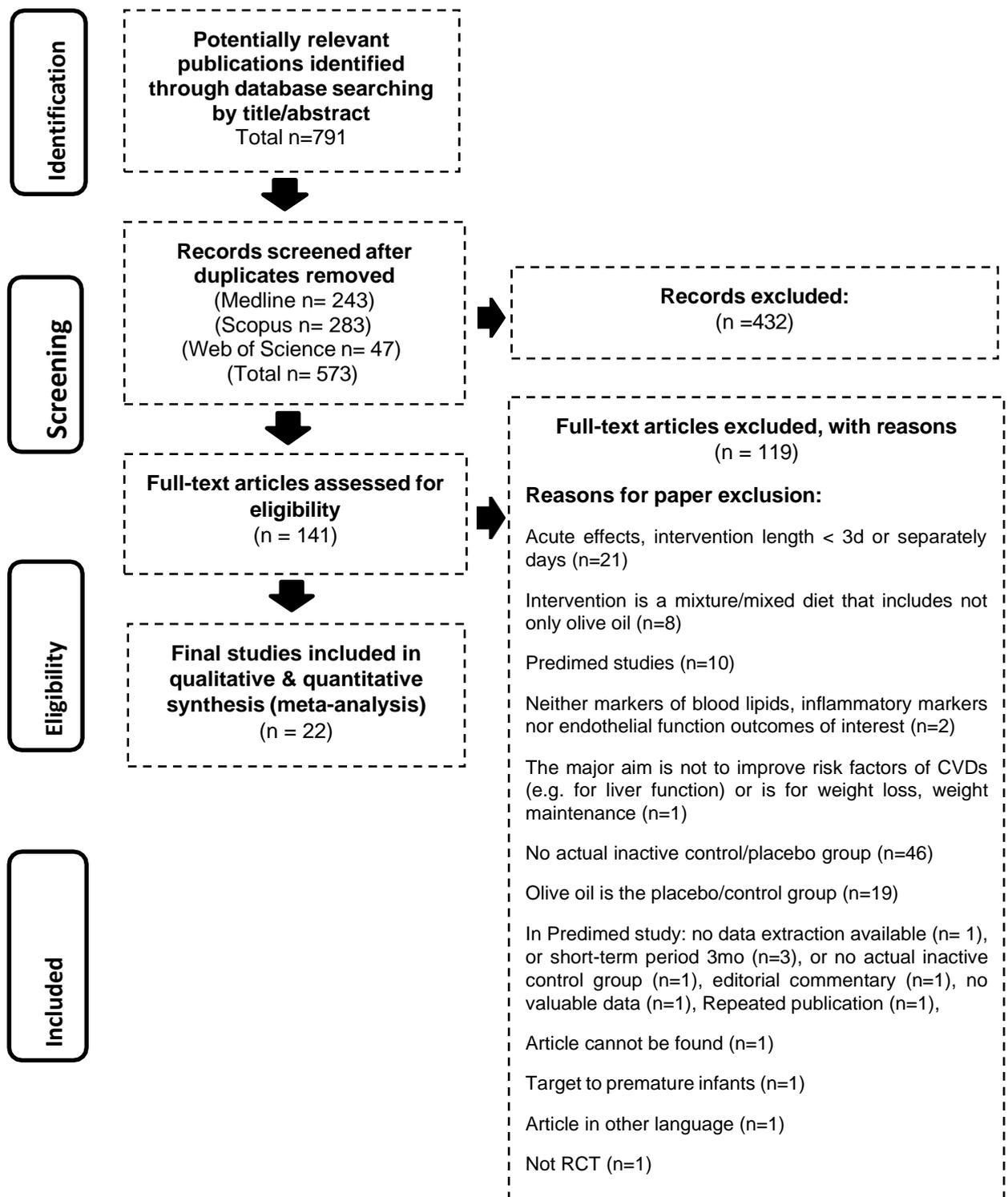
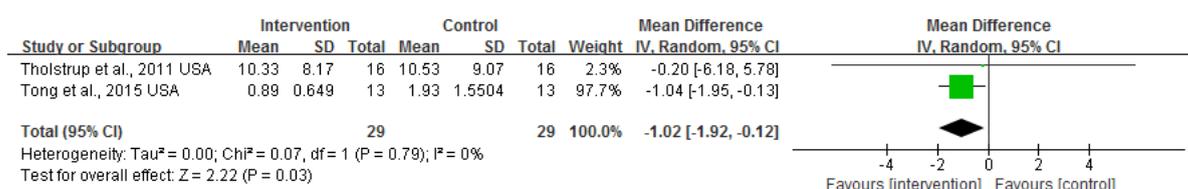


Figure. 4.1. PRISMA flow diagram of selection of studies on olive oil consumption and CV risk factors.

Figure 4.2. Meta-analysis of studies consuming olive oil on plasminogen activator inhibitor-1 (PAI-1) (ng/mL).



4.3.3. Subgroup analysis

Subgroup analysis according to ethnicity was undertaken. Due to limited numbers of studies included in this review, only one study (Voon, Ng et al. 2015) derived from Malaysia and reported that there is no improvement on the cellular adhesion molecule family including E-selectin, sICAM-1 and sVCAM-1. Other studies are all non-Asian population based.

4.3.4. Quality of studies

The methodological quality and risk bias of the RCTs are included in this review. The average retention rate for the trials included in this review was 95.9% for all 22 trials (**Table 4.1**). The Jadad rating score was used to score the randomized trials from 1 to 5 points (Jadad, Moore et al. 1996, Berger and Alperson 2009). Quality scores were assigned with one point each for whether randomization and blinding were used, participant withdrawals and dropouts, random number generation, and the method of blinding on a scale from 0 to 5 (Moher, Pham et al. 1998). 15 studies achieved the Jadad score of 5 while 7 studies achieved the Jadad score of 4.

Table 4.3 reported the quality scores of the included RCTs. All studies were randomized, but only two studies presented the appropriate methods such as generated random order (Maki, Lawless et al. 2015) and a computer-based randomization (Voon, Ng et al. 2015). Most RCTs (n=20) described methods for monitoring or verifying participant compliance. 11 studies had no dropouts and reasons of the dropouts for other studies were mostly due to personal issues except one trial (Kris-Etherton, Pearson et al. 1999) which reported dropouts that were due to the long length of the intervention period (**Table 4.3**). In addition, most studies (n=20) did not report allocation concealment of treatments.

4.4. Discussion

4.4.1. Principal findings

Synthesis of data available from 22 RCTs with 860 participants for an average 5 weeks in the present systematic review and meta-analysis suggested that the interventions with olive oil or olive oil supplementation provide small beneficial effects on three distinct cardiovascular risk factors, which are PAI-1 and tPA while other cardiovascular risk factors are unaffected by olive oil interventions. However, significant result of tPA was abstracted from single study so that the result was not included into the meta-analysis. Overall, the presently available data are too small for drawing a solid conclusion.

4.4.2. Agreements with prior systematic reviews

PAI-1 is well established as a contributing pro-thrombotic and anti-fibrinolytic factor in CV events (Huber, Christ et al. 2001, Tofler, Massaro et al. 2016). t-PA antigen also was more convincingly demonstrated than PAI-1 to be an independent risk factor for CVD (Ridker, Brown et al. 2004). In this meta-analysis, a decreased PAI-1 (mean difference: -1.02; CI: 1.92 to -0.12, $p=0.03$, $n=2$ trials) or t-PA antigen concentration (mean difference: -5.85; CI: -8.72 to -3.43; $p<0.001$; $n=1$ trial) were seen indicating that corresponds to an increased circulating fibrinolytic activity (Tofler, Massaro et al. 2016). While increasing PAI-1 levels (mean: 29.1 ng/ml) with CVD incident were associated with a worse risk factor profile, similar differences across quartile of t-PA were observed (mean: 12.0 ng/ml) in the Framingham Heart study (Tofler, Massaro et al. 2016). Elevated plasma PAI-1 levels are also associated with endothelial dysfunction (Lyon and Hsueh 2003). Notably, factors such as levels of TG and VLDL-C stimulate plasma PAI-1 levels (Bilgic Gazioglu, Akan et al. 2015). In the present meta-analysis, TG was shown to have a tendency of reduction which also implied the decrease in PAI-1. However, increased plasma concentrations of TNF-alpha are not positively associated with the decreased PAI-1 level in the present review.

4.4.3. Strengths and limitations

The strengths of the current findings include a rigorous methodology in the systematic review of the literature and meta-analysis, including prospective registration and the use of the Cochrane Risk of Bias tool to evaluate the quality of evidence. The low levels of heterogeneity surround the results.

The small number of included studies ($n=22$) limits this meta-analysis in the first instance. Most outcome parameters such as SBP, DBP, ApoA-1, Apo B and CRP were collected in

a low number of studies with a relatively small sample size except blood lipids outcomes. This may at least in part explain the difficulty to model a link between biomarkers of cardiovascular via synthesizing the corresponding data from trials with heterogeneous designs. As for some outcome variables such as vWf, sICAM-1, sVCAM-1, IL-6, IL-8, fibrinogen, ET-1, E-selectin, only one or two studies could be included. A small number of participants in total (n = 860) for the 24 risk factors but only three variables are improved after olive oil intake. Due to the small numbers, outcome PAI-1 are of considerable heterogeneity (0%) and the present of improvements on outcomes are only from one or two studies. Since the effect sizes were found to be small, the results should not be over interpreted. Neither the risk of bias nor subgroup analysis can be fully assessed because of the small number of studies.

Trials varied regarding study design, e.g., length of intervention, amount and type of olive oil used, classification of alternate source of fat ("control"), number of participants. In addition, most of study designs prescribed the intake of extra virgin olive oil (EVOO), refined olive oil, raw olive oil provided by the investigators or researchers in pre-defined amounts (e.g., 13.3 g/day, 15 ml /day) to ensure a regular intake in combination with the habitual diets.

In addition, PAI-1 gene expression in vitro and in vivo is induced by a huge amount of triggers including TNF- α and PAI-1 which was found to be correlated positively with TNF- α (Bilgic Gazioglu, Akan et al. 2015). Nevertheless, in the present review TNF- α was shown to adversely correlate with PAI-1. This is probably due to lack of adequate studies to correctly address the value of both outcomes. Furthermore, changes in *hs*-CRP, LDL-C and TG did not differ between olive oil interventions and respective controls/placebos in the present meta-analysis, which might be explained by the low number of study participants enrolled in the RCTs assessing these parameters. Taken together, these limitations implicate a cautious interpretation of the results of our meta-analyses.

4.4.4 Scientific analysis of findings

A number of potential mechanisms are probably responsible for the findings of this review. Firstly, vascular reactivity is affected by food consumption (Kay, Kris-Etherton et al. 2006). Anti-oxidant compounds in food can limit oxidative damage and restore endothelial function making atherosclerotic events slow down (Tousoulis, Psaltopoulou et al. 2015). Therefore, polyphenol consumption has been associated with low mortality rates caused by CV events (Tresserra-Rimbau, Rimm et al. 2014). Furthermore, endothelial function was shown to be

improved by anti-oxidant and anti-inflammatory polyphenols (Zern and Fernandez 2005). Hence, the phenolic compounds such as oleuropein, tyrosol and hydroxytyrosol, antioxidants present in olive oil might mediate the beneficial effects on FMD in the present review. Oleic acid, as another potential health-promoting ingredient of olive oil, in olive oil was demonstrated to benefit cardiovascular risk factors (Bermudez, Lopez et al. 2011). Beneficial effects of MUFAs such as oleic acid on CV risk factors have been consistently reported in meta-analyses and meta-regressions (Schwingshackl and Hoffmann 2012, Martinez-Gonzalez, Dominguez et al. 2014, Schwingshackl and Hoffmann 2014) although the data available at present are still ambiguous. Polyphenols derived from olive oil are characterized as antioxidants, antiplatelet agents, and anti-inflammatory agents. Such functions may regulate haemostasis by directly inactivating PAI-1 and the inactivation of PAI-1 may contribute to the pro-fibrinolytic effects (Cale, Li et al. 2010).

4.4.5. Implications for health and future research

This meta-analysis delivers small evidence for the positive effects of olive oil on markers of inflammation and endothelial function including PAI-1, FMD and tPA. Further studies need to be found and researched concerning olive oil consumption on different ethnicities.

4.5. Conclusion

The available evidence on the effects of olive oil on CV risk factors reported that olive oil consumption improves biomarkers including PAI-1 and tPA. Further studies regarding olive oil consumption and cardiovascular risk factors need to be researched.

Research questions raised from the present chapter 4:

The work described in this chapter raised the following questions:

1. What cardiovascular risk factors and blood biomarkers will be changed after the consumption of olive oil consumption among Caucasians, mostly British and East Asians, mostly Chinese in Newcastle upon Tyne?
2. Dose the effect of olive oil on cardiovascular risk factors similar between different ethnic population living in the Northeast England?

These questions will be addressed in **Chapter 5**.

Table 4.1. Characteristics of randomized controlled trials included in systematic review and meta-analysis.

Author/ year; country of origin	Study design	Ethnicity (%)	Age±SD/ SEM	Sex (%male)	Mean BMI (kg/ m2)	Healthy status	Baseline sample size	Intervention length	Intervention	Dose and frequency	Control	Retention rate	Feasibility & acceptability	Total Jadad score
Atefi et al., 2018 Iran	RCT, parallel	N/A	58 ± 6y	0	28.6±12. 2	T2DM	OO n=27; SO n=27	4 wks	OO	30 g/d	Sunflowe r oil	95	Good	5
Binkos ki et al., 2005 USA	RCT, double- blinded, crossov er	N/A	46.2±SE M8.2y (25-64y)	38.7	26.1±SE M0.3	Moderat e hypercho lesterole mia	Total n=31	4wks*3	OO diet	17.2% of oilve oil in the OO diet daily	Average American diet (AAD)	100	Good	5
Cicero et al., 2009 Italy	RCT, double- blinded, parallel	Caucasia n	50 ± 1 y	50	Corn: 25.7 ± SEM0.8; Olive:25. 7 ± 1.1	Moderat e hypercho lesterola emia	OO n=11; corn oil n=11	45d	EVOO diet	Protein: 16.2%; Carbohydr ates: 54.3%; Fat: 28.9%; SFA: 8.1%; PUFA: 3.8%; MUFA:	Corn oil diet in identical cans as OO	100	Good	5

										16.8%; Cholesterol : 195.2%				
de Oliveira et al., 2017 Brazil	RCT, parallel, double-blind	N/A	67.4 ± 5.15y (60 - 95 y)	29	33.5±3.87	Obese or overweight	n=79; OO n=24; Flaxeed n=26	90d	OO	30 mL/d	Flaxseed oil (FO)	96	Good	5
Engel and Tholstrup, 2015 Danmark	RCT, double-blinded, crossover	N/A	40.4 ± 14.8y	30	23.5 ± 2.5	Healthy	Total n=50	5 wks*2	Refined OO baked into a bread roll	13.3 g/d	16.6 g butter baked into a bread roll	94	Good	4
Galvão Cândido et al., 2018 Brazil	RCT, double-blind, parallel	N/A	27.0 ± 0.9 y; (19–41 y)	0	30.1±SEM0.6 (26 - 35)	Healthy	OO n=33; SO n=28	9 wks	EVOO with breakfast	25 mL/d	25 mL of soybean oil with breakfast	67	Good	5
Jiménez-Gómez et al., 2009 Spain	RCT, crossover	Caucasians	N/A	100	N/A	Healthy	Total n=20	4wks	OO breakfast, & bread, tomato, skimmed milk, hard-boiled egg	36% MUFA; 25% CHO; PUFA: 4%; α-LNA: 0.7%	Butter breakfast, based on the consumption of butter, wholemeal bread,	100	Good	5

											hard-boiled egg and whole milk			
Kontogian ni et al., 2013 Greece	RCT, crossover	N/A	25.6 ± 5.9y (18-35y)	21.6	21.9±2.5	Healthy	Total n=37	6 wks	OO	15 mL/d (13.8 g/d)	FO	100	FO was barely accepted causing the high drop-out rate & worse odour and flavour of the intervention oil	5
Kris-Etherton et al., 1999 USA	RCT, crossover, double-blinded	N/A	21–54 y (x: 34 y)	41	20–27	Healthy	Total n=24	24d	OO diet	Carbohydrate: 50%; Protein: 16%; Fat: 34%; SFAs: 7%; MUFAs: 21%; PUFAs: 6%; Cholesterol : 200%	AAD	91.67	Excellent.	5

Khaw et al., 2018 UK	RCT, parallel	White: 95.8; Non-white: 3.2	59.9 ± 6.1y (50-75 y)	33.1	25.1±4.2	Healthy	OO n=32; Butter n=33	4 wks	EVOO with usual diet	50 g/d	Butter with usual diet	97	Good	5
Lichtenstein et al., 1993 USA	RCT, double-blinded, crossover	N/A	61 ± 13y; 44-78y	46.7	27.4±4.4	Hypercholesterolemic (LDL-C levels >130 mg/dL); postmenopausal	Total n=15	32d *4	OO diet	7% of olive oil in the diet daily	Corn oil diet	100	Good	5
Maki et al., 2015 USA	RCT, double-blinded, crossover	Non-Hispanic white: 75.9; Non-Hispanic Black/African American: 14.8; Non-Hispanic Other: 5.6; Hispanic/	53.8± SEM1.3y	35.2	28.2± SEM0.5	Healthy (active, nonpregnant, nonlactating)	Total n=57; CO/EVOO n=29; EVOO/C n=28	21d (3wks)	Extra-virgin OO (EVOO)	54 g (4 tablespoons) 3 servings of muffin, dinner roll, yogurt daily	Corn oil	94.7	Excellent. CO & EVOO treatments, respectively, was 96.2% (0.5%) & 97.5% (0.2%)	5

		Latino: 3.7												
Namayan deh et al., 2013 Iran	RCT, parallel	N/A	41.7 ± 8.3 y	50	27.4±3	Hyperch olesterol emic (choleste rol >200 and ≤240 mg/dl)	Total n=48; OO n=24; Sesame oil n=24	4 wks	Refined OO	60 g/d (4 table spoons aprox.)	Sesame oil	100	Good	4
Nielsen et al., 2002 Denmark	RCT, double- blinded, crossov er	N/A	23.9y (20-28y)	100	22.9 (18.4-27)	Healthy (non- smoker)	Total n=18	3wks*3	OO diet	50 g per 10MJ of OO incorporate into the diets	Sunflowe r oil diet	100	Good	4
Oliveras- Lopez et al., 2013 Spain (Malaga)	RCT, double- blinded	Spanish	81.7± 6.3 (65- 96 y)	24.2	26.3±3.1	Healthy elderly	Total n=62; CG n=39; OG n=23	6 wks*2	Polyphenol- rich raw EVOO enriched with usual dietary habits	64.8g/d of EVOO (50ml/d in raw form)	Usual diet	100	Good	4

Pedersen et al., 2000 USA	RCT, double-blinded, crossover	N/A	24y (20–28 y)	100	23 (18-27)	Health non-smoker	Total n=18	3 wks*3	EVOO diet	50 g of oil per 10 MJ incorporated into a constant diet	Chemically refined sunflower oil diet	100	Good (high compliance)	5
Perona et al., 2004 Spain	RCT (sequential dietary intervention), parallel	N/A	84.0±7.4 y	32.3	28.8±5.2	Medically treated hypertensive elderly & normotensive (NT) elderly	Total n=62 HT n=31; NT n=31	4 wks	VOO diet	60g/d	Sunflower oil diet	100	Good	4
Sirtori et al., 1992 Italy	RCT, crossover	N/A	N/A	N/A	Male: >25; Female: >23.5	T2DM; hypercholesterolemia	Total n=12	6 wks	OO with monounsaturated diet	Energy: 1600-2000; Protein: 18-19; Carbohydrates: 51-52%; Total fats: 27-30%; SFA: 5%; MUFAs: 18%; PUFAs:	Corn oil with linoleic acid-rich diet	100	good	4

										3%; Cholesterol : 17-19%				
Tholstrup et al., 2011 USA	RCT, double-blinded, crossover	N/A	29.6 ± 10.3y (19 - 64 y)	100	22.9 ± 2.5	Healthy	Total n=43	3 wks*3	OO incorporated into buns and cakes	17% of the energy intake	Palm olein incorporated into buns and cakes	74.4	Good	4
Tong et al., 2015 USA	RCT, parallel	Total: White: 73.8; Black: 26.2	58 ±1y	Total: 23.8; OO: 30.77; Naive: 15.38	OO: 26.3± SEM1.3; Naive: 24.9± SEM1.2	Healthy	OO n=13; Naive n=13	4wks	OO capsules (filtered air)	3g/d (three times of 1g capsules/d of OO)	No supplements (naïve) (filtered air)	100	Good	5
Venturini et al., 2015 Brazil	RCT, parallel	CG: White:69.04, Not White: 31; OO: 84.61, Not White: 15.38	CG: 51.7±8.2 ; OO:51.9 ±7.4 (Median ±SD)	CG:21.4 2; OO:38.5	CG: 37.7±17.64; OO:32.5 ±15.82	Healthy	Total n=55; CG n=42; OO n=13	90d	EVOO	10mL/d	Usual diet	100	Good	5

Voon et al., 2015 Malaysia	RCT with a 3 × 3 Latin Square, crossover	Malaysian (Asian)	30.1±8.3 y	20	23.1±3.7	Healthy	OO n=15; Naïve n=15	5 wks	VOO incorporated into a high-protein Malaysian diet	Fat: 31%; OA: 19.1%; SFA: 6.6%; PUFAs: 4.4%; MUFAs: 20.1%	Virgin palm olein incorporated into a high-protein Malaysian diet	100	Good	5
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Table 4.2. Pooled estimates of effect size for the results of olive oil interventions compared to respective controls.

Outcome Parameter	Mean Difference	95% Confidence Interval	p-Value	No. of Studies	Sample Size	I ² (%)
PAI-1 (ng/mL)	-1.02	1.92 to -0.12	p = 0.03	2	58	0
FMD (%)	2.3	0.05 to 4.55	p = 0.04	1	26	N/A
tPA (ng/mL)	-5.85	-8.27 to -3.43	P < 0.00001	1	26	N/A

Table 4.3. The quality scores of the included RCTs.

References	Generation of Random method clarification	Method of monitoring subject compliance	Drop-outs reasons clarification/withdraw reasons	Allocation concealment of treatment
Atefi et al., 2018	Balanced block method	24-h food records	Fail to follow the diet; insulin need; need for blood lipids lowering drugs	N/A
Binkoski et al., 2005 USA	Random, balanced order sequence	Body weight measurements and a dietary assessment questionnaire	No dropouts	N/A
Cicero et al., 2009 Italy	N/A	7-day nutritional diary	No dropouts	N/A
de Oliveira et al., 2017	Random: Order of entry	N/A	N/A	N/A
Engel and Tholstrup, 2015 Danmark	Random order	3-d dietary records	Personal practical problems; Moving to another part of the country	N/A

Galvão Cândido et al., 2018	Block randomization	The return of the packages	Secondary pathological events; personal reasons	N/A
Jiménez-Gómez et al., 2009 Spain	N/A	Diary records	No dropouts	N/A
Kontogianni et al., 2013 Greece	N/A	Total fatty acid analysis in erythrocyte membranes; 3-day food records	Organoleptic characteristics; exacerbation of irritable bowel's symptoms; protocol misunderstanding	Yes: a lack of blinding due to flavour
Kris-Etherton et al., 1999 USA	Random, balanced order sequence	Body weight measurements and a dietary assessment questionnaire	Long length of the study; relocated to different geographical areas	N/A
Khaw et al., 2018	Computer generated allocation	Open-ended self-reported comments	Mind change; personal reasons;	N/A
Lichtenstein et al., 1993 USA	Randomized order	Plasma fatty acid check	No dropouts	Yes
Maki et al., 2015 USA	SAS-generated random with the seed number recorded	The returned uneaten portion of food items	An intolerance to eggs; financial reasons; the investigator's discretion	N/A
Namayandeh et al., 2013 Iran	N/A	N/A	No dropouts	N/A
Nielsen et al., 2002 Denmark	N/A	Food records and plasma analysis of TAG and cholesterol fatty acids confirmation	No dropouts	N/A
Oliveras-Lopez et al., 2013 Spain (Malaga)	N/A	Semi-quantitative food frequency questionnaire	No dropouts	N/A

		(FFQ); an individual 7-day weighed food diary		
Pedersen et al., 2000 USA	N/A	The fatty acid composition of cholesteryl esters in plasma	No dropouts	N/A
Perona et al., 2004 Spain	N/A	A 24h recall and food frequency questionnaires	No dropouts	N/A
Sirtori et al., 1992 Italy	N/A	A 1-week dietary recall	No dropouts	N/A
Tholstrup et al., 2011 USA	Randomized order	3-d food records	A baseline cholesterol concentration .5.2 mmol/L	N/A
Tong et al., 2015 USA	N/A	3-day food records	No dropouts	N/A
Venturini et al., 2015 Brazil	N/A	Boxes of capsules were handed out and return to count the remaining capsules or bottles	No dropouts	N/A
Voon et al., 2015 Malaysia	Computer-based procedure	N/A	No dropouts	N/A

CHAPTER 5

The effects of extra virgin olive oil or butter on blood pressure and other cardiovascular biomarkers In Caucasians and East Asians male adults in the UK:

A study for a single centre, randomized, controlled, open label, crossover trial

5.1 Introduction

Evidence from prospective studies shows that the Mediterranean diet with olive oil as the predominant fat source is associated with numerous health benefits (Visioli, Franco et al. 2018). These benefits are in part attributed to its high monounsaturated fatty acid content as well as to bioactive components found in olive oil. A number of studies have tested the effects of consuming olive oil on intervention trials. However, most studies on the Mediterranean diet and on the health effects of olive oil have included mostly Caucasian individuals (Mazzocchi, Leone et al. 2019).

Diet-related disparities, reflecting differences in diet, as well as in the incidence, prevalence, mortality, and the burden of disease between and within specific population subgroups, have been long recognised (Satia 2009). No individual is identical to another; there is significant variation in individual constitution, lifestyle, genetics, diet, health, and other factors. Ancestry and ethnicity are important determinants of health. Often, the disease risk profile of some ethnic groups within a population differs from that of the majority population – sometimes in favour, but mostly to the detriment of minority populations. Certain countries such as the UK or the USA have become increasingly diverse over the last century; in such countries 20-30% of the population make up ethnic minority groups. In spite of the importance of ethnicity, a lack of adequate representation of racial and ethnic minority populations in intervention trials has been identified; this is recognised as a limitation of the generalizability and potential public health impact of these interventions (Haughton, Silfee et al. 2018). Therefore, caution against generalising results from predominantly white study populations to other racial/ethnic populations should be emphasised since ethnicity is a well-known effect modifier, this is a variable for which the effect of the intervention varies across different levels of the variable (Kris-Etherton, Petersen et al. 2020). In the UK the Chinese ethnic group or “British Chinese” migrated from former British colonies, such as: Hong Kong, Malaysia, Singapore, Canada, Australia, New Zealand and Mauritius, with lower numbers from mainland China and Taiwan. Chinese communities are found in many major cities such as London, Glasgow, Manchester and Newcastle. There are well established Chinatowns in London, Manchester, Birmingham, Newcastle and Liverpool.

The North East of England, is characterised by high rates of obesity, poor health (NHS 2020), especially cardiovascular disease, which account for 24% of all deaths (Public Health England 2019). Cardiovascular risk factors such as overweight and obesity levels in the North East currently rank third lowest for men but second highest for women. Cardiovascular diseases including heart disease, strokes and other related conditions are one of the biggest causes of ill health and early deaths in the North East of England. Each year over 6,700 people die from CVD across the region, representing one quarter of all deaths (Public Health England 2019). Approximately 2000 of these deaths occur in people under 75 years of age. Many of the risk factors for CVD including high blood pressure, obesity, smoking, physical inactivity, excessive alcohol consumption and poor diet, are more common in the North East region. In 2015 and 2017, the rate of premature CVD mortality in the North East was the second highest of all the English regions and significantly higher than the national rate (Public Health England 2019). There is an imperative need to reduce the risk of developing cardiovascular diseases by developing strategies to promote healthy eating, physical activity, smoking cessation and weight loss.

A study reported that there was much lower prevalence and mortality of stroke, and higher prevalence and higher mortality rates from CHD among Chinese immigration (Gong and Zhao 2016). For Chinese immigrants in the North East of England, however, there is a lack of literature of evidence exploring and showing whether or not Chinese citizens, or East Asians population living in the North East of England experience either cardiovascular diseases or cardiovascular risk factors.

5.2 Rationale of the clinical trial

No dietary interventional studies have directly compared different ethnic groups such as Caucasians and East Asians although a number of studies have been undertaken to evaluate the impact of olive oil in a number of cardiovascular risk markers. In addition, few studies have evaluated blood pressure, one of the most important risk factors of cardiovascular disease, using ambulatory blood pressure measures which have a stronger association with CVD outcomes than clinic blood pressure (Piper, Evans et al. 2015). Similarly, few studies have reported on the impact of olive oil on blood markers of endothelial function.

The evidence from the survey study undertaken as part of this PhD and reported in **Chapter 2** suggested that olive oil (OO) intake is positively associated with PREDIMED score (MDPS) as well as MD acceptability and inversely associated with perceived barriers to healthy

eating (PBHE). OO is less popular among Asians than Caucasians, however the acceptability of OO consumption is relatively high in both ethnicities.

Systematic reviews and meta-analysis of the relevant literature were undertaken to inform the design of this olive oil human trial. The evidence from the systematic reviews and meta-analyses of relevant literature (**Chapter 4**) suggested that olive oil consumption with daily consumption ranging between 13.3 and 64.8 g, significantly reduced important biomarkers of cardiovascular function such as PAI-1 and tPA. There was no significant evidence of a reduction on TC, LDL and BP. However, DBP and TC are more likely to be improved by olive oil consumption. Only few studies, however, have assessed 24-hour BP after extra virgin olive oil intake between Caucasians and East Asians. However, the evidence of different effects of OO consumption on emergent circulating biomarkers such as CRP, sICAM-1, sVCAM-1, PAI-1, sP-selectin, sE-selectin and IL-6, in East Asians and Caucasians is currently less examined in the literature. Novel biomarkers such as syndecan-1 in the present study have not been studied in relation to whether they are improved by olive oil consumption. Furthermore, levels of circulating markers of inflammation and atherosclerosis including interleukin 6 (IL-6), soluble intercellular adhesion molecules (sICAM-1), soluble vascular adhesion molecules (sVCAM-1), E-selectin and P-selectin have been only scarcely studied on the impact of the effects of extra virgin olive oil between Caucasians and East Asians too.

On the basis of these results, therefore, this study aims to determine and compare the beneficial effects of extra virgin olive oil (EVOO) and butter after 6-week interventions on resting blood pressure, 24 hours ambulatory blood pressure, blood lipid profile including TG, TC, LDL-cholesterol, HDL-cholesterol, circulating markers of inflammatory cytokines including CRP, IL-6, PAI-1, adhesion molecules such as sE-selectin, sP-selectin, sICAM-1, and sVCAM-1, as well as novel biomarkers such as syndecan-1 in Caucasians and East Asians who live in the North East of England.

The hypothesis of this human clinical trial chapter is to test if the cardiovascular risk factors will be positively effective by EVOO intake in healthy participants both ethnicities and EVOO will have different levels of positive effects on cardiovascular risk factors between healthy Caucasians and East Asians. A comprehensive study examining liquid extra virgin olive oil (EVOO) on both traditional and novel CVD risk factors is lacking in the literature.

5.3 Method and Materials

The study was subject to ethical review by the Northumbria University Ethics Committee (**Project ref: 10527**) and was given a favourable ethical opinion to proceed. Informed signed consent was sought from each participant.

The study protocol was written in accordance with the Standard Protocol Items: Recommendations for Interventional Trials (SPIRIT) guidelines (Chan, Tetzlaff et al. 2013) and Consolidated Standards of Reporting Trials (CONSORT) guidelines (**Figure 5.1** and **Appendix D8**) (Dwan, Li et al. 2019). The study was conducted at Northumbria University facilities. In total 34 male participants who live in Newcastle upon Tyne of the North East of England completed the intervention, the participants were statistically analysed as a full sample size as well as being split into Caucasians (n=18) and East Asians (n=14). In addition to traditional biomarkers, there are a number of novel biomarkers that have shown clinical potential to predict the incidence of CVDs. These novel biomarkers are involved at various stages of the atherosclerosis process.

CONSORT 2010 Flow diagram

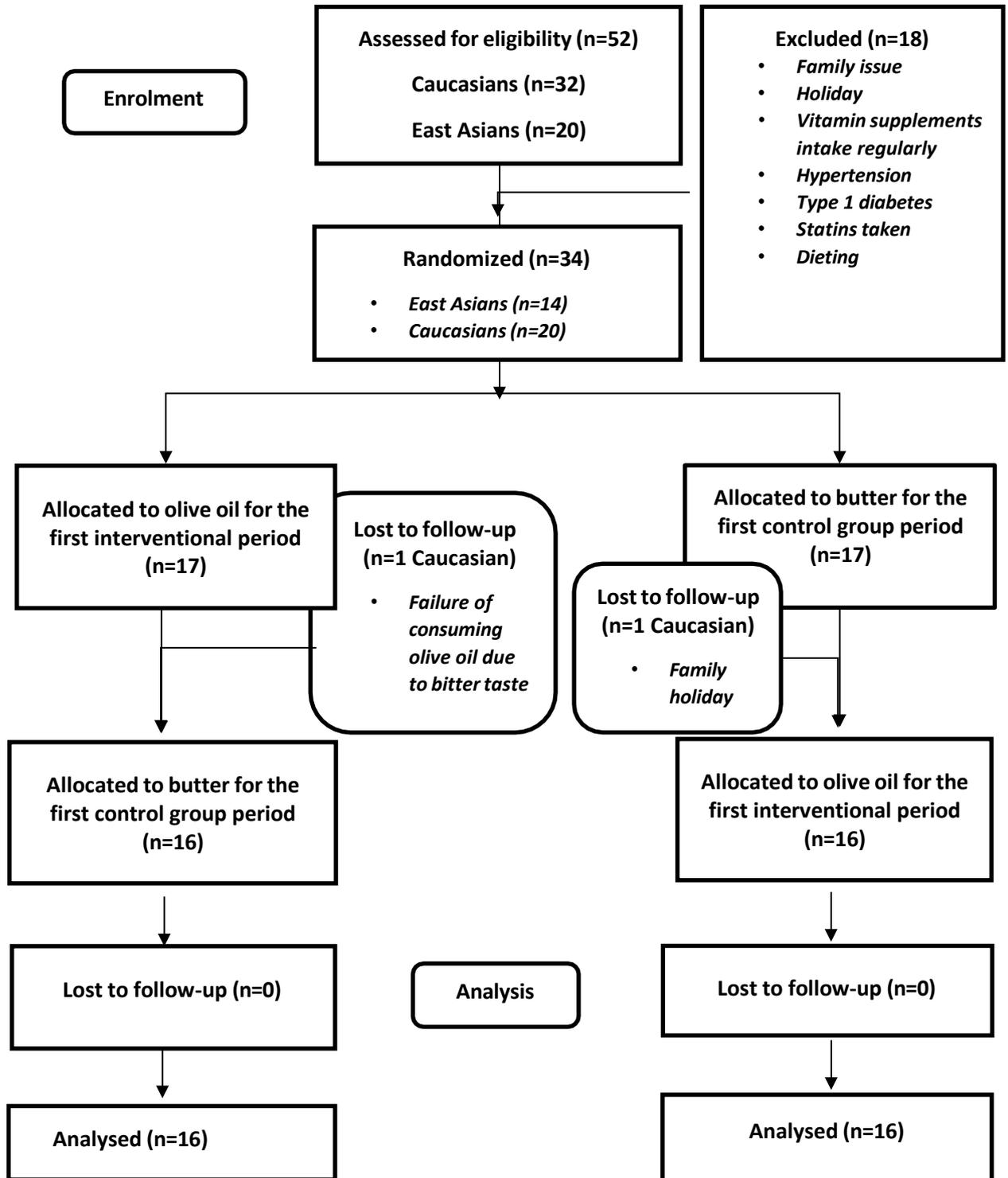


Figure 5.1 CONSORT Flow diagram for reporting of the results of the human clinical trial

5.3.1 Trial design

This study is a single-centre, two-arms, crossover randomised controlled superiority trial. A crossover design was chosen in order to evaluate within- and between-subject effects of the interventions tested so as to eliminate between-subject variability. A crossover design offers two advantages over a parallel-group RCT: (1) the influence of confounding covariates are reduced because each participant serves as their own control; and (2) statistical power is higher and required sample size to detect meaningful effects is lower (Stephen 2002). The protocol for this trial has been registered at **Clinical Trials** (<https://clinicaltrials.gov/>) **registration: NCT04187638**).

This study involved 4 study visits over a 6-week period plus a screening visit prior to the study visit 1. During the pre-screening visit, participants received a participant information sheet, consent form, a physical activity questionnaire, plus a medical and lifestyle questionnaire. Two questions in the lifestyle questionnaire were related to OO intake (use of OO as a main fat for cooking or dressing and using how many tablespoons of OO). Participants were asked to complete a 3-day dietary record including 2 weekdays and 1 weekend day to take into account any differences in nutrient intakes during weekdays and weekends at pre-screening visit prior to future visit 1. Participants were recommended to record their dietary intake with the instructions attached by researcher during the first two-week intervention period and were asked to repeat their own habitual dietary pattern (on breakfast and evening meals) to match up the nutrition intake and energy intake, and to maintain physical activity pattern throughout the rest of the whole study in order to limit dietary intake as well as body weight variability to remain unchanged during the whole clinical study. OO and butter dose was designed to provide approximately 8% to 11% of energy from fat. A comparable energy level was maintained during the two subsequent phases and a 3-day food record was completed before bringing along with to future visits.

On each study visit, participants' blood pressure, anthropometric of body composition and physical strength measurements were collected. In addition, fasting urine and venous blood samples were obtained. Body mass index (BMI) was calculated as weight (kg) divided by height squared (m²), grip strength, body fat and resting blood pressure, ambulatory blood pressure monitoring were obtained following standard protocols (i.e., without shoes or wearing heavy garments) and a fasting venous blood sample (18ml in each visit) was

collected by trained researchers. Therefore, a total of 72ml of blood per individual was collected throughout the entire study. Fasting spot urine samples were also collected into a flask. The outcomes for this study included both resting and 24-hour ambulatory systolic (SBP) and diastolic blood pressure (DBP); urine samples to assess nitric oxide production; blood biomarkers such as cholesterol, triglycerides, LDL, HDL, sICAM-1, sVCAM-1, CRP and IL-6 were analysed from the blood plasma samples. The plasma and urine collected was also used to identify novel markers of dietary intake. Plasma and urine analyses did not commence until the full intervention study was complete, and all samples from each subject were analysed within one batch to reduce inter-batch variation.

Butter was chosen to be the control group since butter is one of the most common fat-based foods that British citizens consume daily. The same brand of butter and extra virgin olive oil were purchased from Tesco - unsalted Block Butter (0.03g of salt/30g butter) and Filippo Berio Organic Extra Virgin Olive Oil were chosen throughout the trial to prevent confounding effects from butter containing salt (0.45g of salt/30g butter). Over-consumption of salt causes raised blood pressure and consume less than 6g (0.2oz) of salt a day, which is about a teaspoonful was the goal to reduce blood pressure (NHS 2019). The researcher instructed each participant to measure the dose of extra virgin olive oil (EVOO) to be consumed per day by spoon as well as the amount of unsalted block butter mixing with the can of soup consumed at lunchtime as a lunch meal replacement. During two-week washout period, participants were asked not to consume the olive oil and butter provided or any other types of olive oil.

5.3.2 Participants eligibility criteria and study settings

Adult, self-reported healthy, adult men (20-64 years of age), of two different ethnicities - Caucasians mostly British and East Asians mostly Chinese, participated in this study. East Asians (including Chinese, Hong Kong, Korean, and Malaysia Chinese) and Caucasians (including local British and Western Europeans such as Croatian, Irish) aged 20-64 years were included and studied. Only male individuals took part in the study. Women were not included as controlling for the potential influence of menstrual cycle would increase the length of this study, making it thus unfeasible within this PhD project. Although the lack of diversity of gender remained an issue in general CVD clinical trials, it is of vital importance

to notice that this trial was conducted among only 34 participants with two ethnicities and a large age range within a limited period of time, which lasts around ten months, therefore in order to complete this trial in a practical way, it is necessary to maintain other controllable aspects (e.g. gender) the same is to keep consistency of the subjects in order to reduce the compounding factors which potentially complex the design of the intervention and affect the trial results.

Participants were volunteers living around Newcastle upon Tyne, Northeast England, UK. Subjects were excluded if they are taking antioxidant supplements; diagnosed and/or taking medications for hypertension (>140/90mmHg), diabetes, high blood cholesterol and heart problems; diagnosed lactose intolerance; taking omega-3 supplements regularly in the last six months, or if they reported being allergic to olive oil.

Participants were recruited via social media including Facebook, LinkedIn and Twitter and other reachable forms of community recruitment including web advertisements, and flyers and posters in local venues from January until November 2019. Participants were asked to attend 1 screening visit and 4 study visits over a 6-week study period held in Northumberland building (NB425) at Northumbria University. These visits were scheduled in the morning and lasted around 30 minutes to one hour. During the study days, participants were asked to refrain from strenuous exercise. Participants returned at the same time point for the study visit every 2 weeks and repeated all of the measurements mentioned above.

5.3.3 Interventions

Participants received two treatments incorporated into their usual diet. In the intervention arm, participants were supplemented with at least 30 ml/day of extra virgin olive oil (EVOO) for two weeks. The control arm received 30 g/day of butter for two weeks. A washout period of 2 weeks in between which participants restrained from consuming either extra virgin olive oil (EVOO) and the butter block followed the intervention arms (**Figure 5.2**). 2-week time of washout period is the minimum washout period which is effective to eliminate the “order” effects which potentially affects the outcome due to the nature of this EVOO intervention. Based on the systematic review of meta-analysis of olive oil in **Chapter 4**, 2-week washout

period was also shown to be sufficiently enough to eliminate the issue of “carry-over” effects between treatments.

The amount of olive oil provided in this study was informed by the systematic review in **Chapter 4**. It was estimated that 30 ml of olive oil for a minimum of 2-weeks would be enough to show effects on the cardiovascular biomarkers.

The nutritional composition of Filippo Berio Extra Virgin Olive Oil (500ml) and Tesco British Unsalted Butter (250g) obtained from Tesco product description and USDA Food Composition Databases is presented in **Table 5.2**. Participants were instructed to replace their usual lunch meal with the OO or butter plus one can of Heinz Cream of Vegetable Soup (400g) or Heinz Potato and Leek soup (400g). In addition, participants also were recommended to eat a piece of fruit (e.g., one banana: 30.75kcal or one gala apple: 70.89kcal) and one slice of white medium bread (90kcal) so that the total calorie intake of the entire lunch meal is similar as the total calorie intake of usual lunch meal. The total energy intake of the provided lunch meal is approximately 600kcal.

Compliance and adverse effects with interventions were assessed by self-report using daily checklists. In addition, body weight and body composition, and handgrip strength were measured on each study-visit as indicators of evidence of maintenance of lifestyles. Participants were also contacted regularly to discuss any problems relating to the supplementation of olive oil and to provide encouragement and support by either emailing or phone call usually at the midpoint of each round of intervention. Self-reported daily checklist of extra virgin olive oil showed a good acceptability and compliance with EVOO. No side-effects of the intervention were reported regarding EVOO and butter consumption.

5.3.4. Randomization and Allocation

Participants were assigned a unique study identification number (ID). Participants were randomized using computer-generated random integer generator software (<http://www.randomization.com>) in blocks of 4, with a 1:1 ratio by the principal supervisor, Dr Jose Lara. This ensured a balanced design with half the sample starting with butter and the other half with olive interventions. A randomisation list was produced for each ethnic

group to minimise potential confounding. The allocation sequence was concealed from the researcher (Fan Liang) using sequentially numbered, opaque and sealed envelopes.

Participants were unaware of the randomization sequence; but given the nature of it, they were not blind to the intervention, once they began each phase of the intervention, they were aware of either the extra virgin olive oil (EVOO) or butter to be consumed.

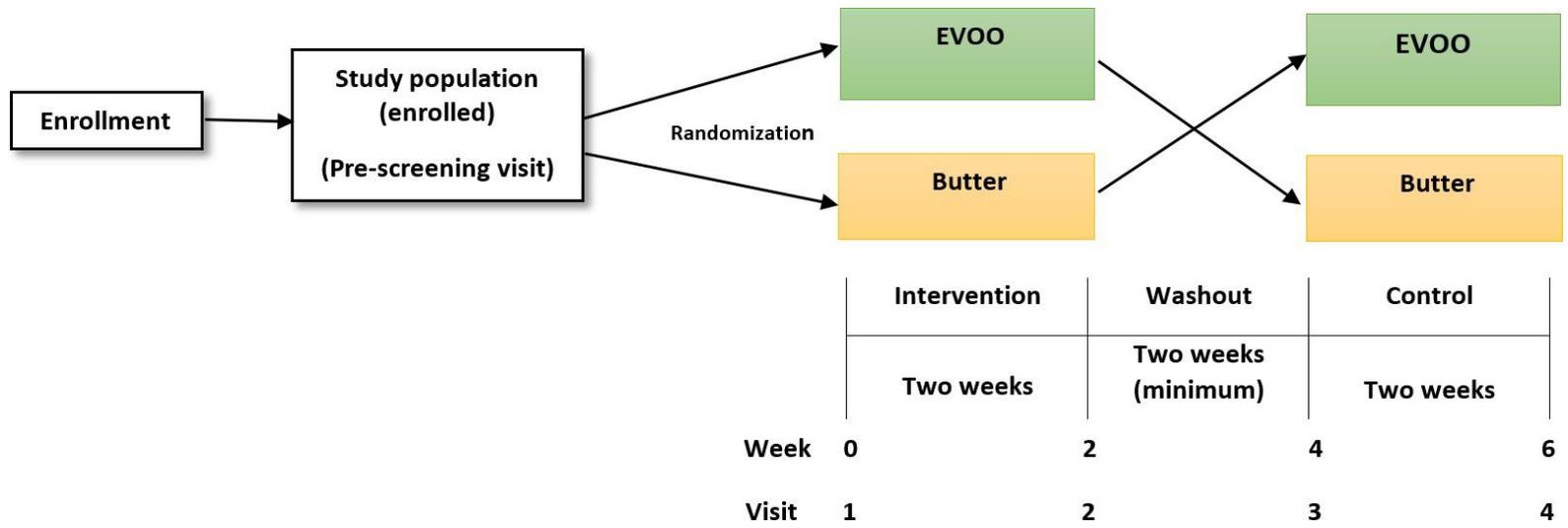


Figure 5.2 Flow diagram of olive oil intervention.

5.4 Outcome measures

The outcome measures of this study are listed in **Table 5.1**. The primary outcome measure of this study is ambulatory blood pressure based on the results of olive oil systematic review and meta-analysis in **Chapter 4** as these outcomes were the most common, representative and typical outcomes assessing the effectiveness of olive oil interventions, while other biomarkers such as these related to endothelial function were poorly studied. Thus, novel biomarkers need to be evaluated in response to the interventions in the clinical trial study.

Secondary outcomes included traditional and novel CV biomarkers (**Table 5.1**). To minimize inter-observer variability all outcome measures in this study were measured by the researcher (Fan Liang) every time. All measurements were carried in a temperature-controlled room in which the ambient temperature was $23\pm 1^{\circ}\text{C}$. To minimize inter-observer variability all outcome measures in this clinical trial were measured by the researcher every time.

Table 5.1. Outcome measures evaluated at baseline, 2, 4 and 6 weeks.

Primary Outcome Measures	
Blood pressure	1. Resting (Laboratory) Blood pressure
	2. 24hr ambulatory blood pressure
Blood lipid	1. TC
	2. HDL-C
	3. LDL-C
	4. Triglyceride
	5. NHDLC
New established biomarker	1. sP-selectin (ng/mL)
	2. sE-Selectin (ng/mL)
	3. Interleukin 6 (ng/mL)
	4. PAI-1 (ng/mL)
	5. CRP (pg/mL)
	6. sVCAM1 (ng/mL)
	7. sICAM1 (ng/mL)
	8. Syndecan-1 (ng/mL)

Table 5.2 Nutrient Content 30 ml of extra virgin olive oil, 30 g of butter, 400g of soup respectively; Data obtained from USDA Food Composition Databases and Tesco product description.

Nutrient (unit)	EVOO	Butter	Heinz Vegetable Soup	Heinz Potato and Leek soup
Total Fat (g)	27.39	2.46	3.4	7.4
Saturated Fat (g)	4.65	1.56	0.2	4.4
Trans Fat (g)	0	0	0	0
Polyunsaturated Fat (g)	3.21	0	0	0
Monounsaturated Fat (g)	19.53	0	0	0
Cholesterol (mg)	0	0	0	0
Iron (mg)	0.07 (0)	0	0	0
Energy (kJ)	1013.4 kJ-246.6 kcal	918.6 kJ – 223.5 kcal	792 kJ-188kcal	832 kJ-196kcal
Carbohydrate (g)	0	0.18	33.2	28.6
Sugars (g)	0	0.18	13	0
Fibre (g)	0	0	3.6	2.2
Salt (g)	0	0.03	2.4	2.4
Protein (g)	0	0.18	4.4	3.2

5.4.1 Resting blood pressure and 24-hour ambulatory blood pressure (ABP)

Resting blood pressure was measured in a quiet room after participants rested for 15 minutes in a seated position with arm resting on a firm surface and feet flat on the floor, using a non-invasive digital automatic blood pressure monitor (Carescape™ V100: GE Healthcare, UK). Blood pressure measurements were taken in the non-dominant upper arm in triplicate, and the average of the last two measurements was used for subsequent analyses.

A non-invasive ABP device (Medical 90217-1Q: Spacelabs, Inc. Richmond, Washington, USA) was utilized to record and measure 24-hour ABP. An ABP cuff were fitted by the researcher, and participants were asked to wear the monitors for a period of 24 hours after completing all other measurements. The ABP blood pressure device that is attached to a belt around participants' bodies, which is connected to a cuff around the upper arm.

All readings were performed on the non-dominant upper arm of the participant on each study day. The monitor was programmed (ABP Report Management System version 3.0.3 Spacelabs, Inc. Richmond, Washington, USA) to inflate automatically, at 30-min intervals

during 0800-2200 and 60-min intervals during 2200-0800, for a total period of 24-hours. Mean daytime and night-time blood pressure were calculated based on measurements taken while participants were awake and asleep, respectively (Yano, Tanner et al. 2019).

Throughout the 24-hours period, participants were instructed to maintain their arm relaxed down the side of their body and kept still until the end of each subsequent measurement. While walking, the participants were required to stop and stand still for a minute until the measurement completed.

5.4.2 Fasting blood sample and urine collection

Fasting blood samples were collected using a BD Vacutainer® Safety-Lok™ blood collection set inserted into the antecubital vein in right arm; blood was drawn into separate 6 mL BD Vacutainer® Heparin Tube tubes (Becton Dickinson). Tubes were inverted 10 times to mix the blood and anticoagulant inside the tube and centrifuged immediately for 15 min at 1500rpm. Plasma samples were instantaneously stored at -80 °C until analysis. Analyses of plasma samples were undertaken after the intervention study was completed and all samples for each participant were analysed without knowledge of the treatments, within one batch to reduce inter-batch variation. Fasting plasma samples (3 x 6ml Li-Heparin tubes) were collected before and after each arm of intervention. A spot (20ml each of spot) urine was obtained in each of the 4 visits in order to assess metabolites (Four in total throughout the study).

5.4.3 Plasma samples analysis

Plasma samples were used for analysis of cardiovascular biomarkers using commercial Elisa kits according to the manufacturer's specifications. A microplate spectrofluorometric reader (Tecan Spark®) was used to measure absorbance at 492 nm on a plate reader (Tecan Spark, Tecan, Switzerland) on each ELISA plate. Viability was calculated and expressed as percent of control. Due to limits in budget, eight biomarkers including P-selectin, interleukin-6, PAI-1, CRP, sVCAM-1, sICAM-1, sE-selectin and syndecan-1, were analysed only at post-intervention, which include study visit 2 and visit 4.

Cardiovascular biomarkers measures included:

Plasma soluble vascular and intracellular adhesion molecules (**sVCAM-1 and sICAM-1**). sVCAM-1 (Product number: RAB0505; Lot number: 1222D0195) (**Appendix D13**) and human sICAM-1 (Product number: RAB0219; Lot number: 0528F0185) (Sigma-Aldrich, St Louis, MO, USA) (**Appendix D14**).

Plasma soluble E-selectin and P-selectin. Human sE-selectin (Cat. No.: RAB0422; Lot number: 0410F0118) (**Appendix D15**) and sP-selectin (Cat. No.: RAB0426; Lot number: 0509F0217) (Sigma-Aldrich, St Louis, MO, USA) (**Appendix D9**).

Plasma pro- and anti-inflammatory cytokines. **IL-6** (Product number: RAB0306; Lot number: 0424F0140) (Sigma-Aldrich, St Louis, MO, USA) (**Appendix D10**).

Plasma concentrations of plasminogen activator inhibitor (**PAI**)-1. (Product number: RAB0429; Lot number: 1016F0312) (Sigma-Aldrich, St Louis, MO, USA) (**Appendix D11**).

C-reactive protein (CRP). CRP (Product number: RAB0096; Lot number: 0925F0283) (Sigma-Aldrich, St Louis, MO, USA) (**Appendix D12**).

Syndecan-1. Human SDC1/syndecan-1 (Product number: RAB0736; Lot number: 0612F0035) (Sigma-Aldrich, St Louis, MO, USA) (**Appendix D16**).

Lipid profile, (including total cholesterol, triglycerides, HDL-C and LDL-C) content in plasma was measured by The Laboratories of the Integrated Laboratory Medicine in Freeman Hospital at Newcastle Upon Tyne. Plasma triglycerides were measured by an enzymatic, colorimetric method with glycerol phosphate oxidase and peroxidase (Trigl, Cobas Roche Diagnostics, Indianapolis, IN, USA). Total plasma cholesterol was obtained by an enzymatic, colorimetric method through the cholesterol esterase/cholesterol oxidase/peroxidase reaction (CHOL2, Cobas Roche Diagnostics, Indianapolis, IN, USA). The plasma concentration of HDL-cholesterol was determined by a homogeneous enzymatic colorimetric assay through the cholesterol esterase/cholesterol oxidase/peroxidase reaction (HDL3, Cobas Roche Diagnostics, Indianapolis, IN, USA). All plasma samples were frozen at -80 °C and kept until analysis.

5.4.4 Other measures and data collected

5.4.4.1 Anthropometric measurements

Anthropometric measurements were taken including height and weight without shoes, which were then used to calculate BMI by dividing body mass (kg) by body height² (m) (kg/m²) (World Health Organization 1995, Madden and Smith 2016).

A stadiometer was used to measure height to the nearest centimetre (cm). Participants were asked to remove their shoes and position their head in the Frankfurt plane position. Participants were positioned looking straight ahead with the lower border of the left orbit and the tragus of the ear lying on the horizontal plane (Raine and Twomey 1994).

Body weight was measured using Tanita scales after participants removed outer garments and any personal objects affecting body weight (keys, coins, jewellery etc.). Participants were barefoot for both height and weight measurements (Kuriyan 2018).

5.4.4.2 Body composition assessment

Bioelectrical impedance analysis (BIA) was performed using a single frequency (50 kHz) device (Body stat 1500, Body stat Ltd; Isle of Man, UK). Estimations of fat in percentage (%) and in weight (kg), lean weight in percentage (%) and (kg), total water in percentage (%) and litre (L) were recorded. Before measurement, participants were rested in the supine position and have their limbs abducted to avoid current shunting. Body stat electrode pads was placed in the middle of the dorsal surface of the left hand just proximal to the metacarpophalangeal joints and left foot proximal to the metatarsophalangeal joints; a second set of electrodes were placed between the distal prominence of the radius and the ulnar styloid and between the medial and lateral malleoli at the ankle (**Figure 5.3**).



Figure 5.3 Bodystat- Electrodes position on hand and foot.

5.4.4.3 Handgrip strength

Handgrip strength was measured using a CAMRY-EH101 hand dynamometer (range 0 to 90kg; accuracy 0.1 kg) (EH101; Camry, Guangdong Province, China). In a standing position, participants hold the dynamometer in their dominant hand (Meng, Wu et al. 2015). The handle of the dynamometer was adjusted if required. When ready, participants were instructed to squeeze the dynamometer with maximum effort and hold for approximately 5 seconds without other body movements (McGrath, Kraemer et al. 2018). The participants were strongly encouraged by the researcher to give a maximum effort. Handgrip strength was assessed in triplicate and the average of all readings were taken as the final score (Lee, Peng et al. 2016, McGrath, Kraemer et al. 2018).

5.4.4.4 Dietary assessment and nutritional analysis

Participants were asked to record their dietary intake for 3 non-consecutive days (i.e., type of foods, preparation and amount of food/drink consumed), two weekdays and one weekend day. The 3-day dietary record was estimated by providing guidance on the estimation of household measures, and food intake record from each participant allowed a nutritional analysis with the aid of the “Micro-diet” software (Micro-diet System, Version 6.4, Salford University, UK) (Rosa, Carmen et al. 2015) (**Appendix D20**).

5.4.4.5 Sample size

This study was powered based on a sample size calculation on the number of participants.

Sample size is calculated using G*Power (<http://www.gpower.hhu.de/en.html>) version 3.1.3 (Program written, concept and design by Franz, Universitat Kiel, Germany) (Faul et al., 2009). T-test for differences between two dependent means was used. Calculations considered a prior power of 0.8, a 0.05 significance level and a 0.5 correlation coefficient between groups.

Sample size calculations were performed for the primary endpoint; this is, change in SBP response. The estimated participants required to allow detection a difference of 6 mmHg between the responses to the intervention and control, based on the systematic review in **Chapter 4**. T-test, difference between two dependent means (matched pairs), a priori: compute required sample size and two tails were chosen. At a power of 0.80, a 0.05 significance level, a sample of 12 in a crossover study allowed to detect a difference of approximately 0.5 standard deviations in SBP (graph shown in **Appendix D17**). We proposed recruiting 15 male adults within each ethnic group in order to allow for a 20% drop-out rate. This sample size is a conservative one, given that in a cross-over study design, the correlation between the groups would be higher which lower the sample size. Therefore, over-recruitment (at least 30 participants) was acceptable and feasible to add weight to this study.

5.5 Statistical analysis

An intention-to-treat analysis was performed (Montori and Guyatt 2001) in accordance with **CONSORT guidelines** (Dwan, Li et al. 2019) to provide an assessment of the practical impact of a treatment.

All statistical analyses were carried out using IBM SPSS Statistics version 24. Data was evaluated for normality of distribution using the Shapiro-Wilk test.

The General Linear Model (GLM) for repeated measures were used to compare within-subject treatment effects. In addition, analysis of variance (GLM univariate) was used to test between subject comparisons. Results (unadjusted and adjusted for covariates) are presented as means (or marginal means) \pm SEM. Adjustment for covariates includes baseline and washout values, the results for blood pressure as well as blood lipids were obtained after baseline, washout, BMI (kg/m²), age (years), sodium (mg/day) intake and energy intake (kcal/day) value. Results for novel biomarkers were achieved after washout, BMI (kg/m²), age (years), energy intake, saturated fatty acids (mg/day) and dietary cholesterol (mg/day) values. Bonferroni correction test for multiple comparisons were implemented. Paired t-tests were used to compare changes from baseline. Analysis was run on the participants as a whole as well as grouped by ethnicity: A p value less than 0.05 is assumed as significant.

5.6. Results

The study was completed during early December 2019. Recruitment for this study was successful and a total of 32 participants took part (**Figure 5.1**). 32 participants completed the entire trial after baseline assessment with a high retention rate (94.2%) and good adherence to the treatments (**Figure 5.1**). The participants reported 100% compliance with interventions, i.e., extra virgin olive oil (EVOO) and butter consumption. Neither body weight, nor other demographic and clinical characteristics changed significantly over the periods of intervention. Research participants experience questionnaire reported that participants either strongly agree or agree with feeling being valued as a participant. No participants reported negative feedback and no adverse effect overall. All but one Caucasian participant reported to be sure they would continue using extra virgin olive oil (EVOO) in the near future after taking part in the study and apart from this participant.

5.6.1 Participants baseline characteristic

The demographic and anthropometric characteristics of the sample studied are shown in **Table 5.3**. In total 32 participants completed the interventional study, age ranged from 20 to 64 years old. The baseline characteristics of participants are presented for the full sample size as well as when these were divided according to their ethnicity into Caucasians (n=18) and East Asians (n=14). Overall, no significant differences were observed between groups at baseline on any of the variables presented in **Table 5.3**. The results shown indicate a sample of mature adults, on average of a normal weight, normal blood pressure and body composition, although some spread was observed, for example in BMI values ranging from 17.7 kg/m² to 31.6 kg/m². Statistical analysis comparing Caucasians versus Asians revealed that these groups were comparable.

Table 5.3 Participants (n=32) baseline characteristic

Baseline characteristics	All participants (n=32)			*Caucasians (n=18)			**East Asians (n=14)			P-value (Sig. 2-tailed)
	Mean	SEM	STD	Mean	SEM	STD	Mean	SEM	STD	
Age (year)	31.22	1.83	10.33	33.72	2.97	12.60	28.00	1.39	5.20	0.122
BMI (kg/m ²)	24.11	0.63	3.59	24.64	0.79	3.36	23.44	1.04	3.87	0.355
Resting SBP (mmHg)	117.60	1.76	9.97	120.32	2.02	8.58	114.09	2.89	10.82	0.079
Resting DBP (mmHg)	68.60	1.95	11.03	67.28	2.38	10.11	70.30	3.29	12.30	0.451
Resting HR (bpm)	69.20	1.77	10.03	67.98	2.37	10.05	70.77	2.71	10.14	0.443
24-hour SBP (mmHg)	120.70	1.37	7.78	122.13	2.16	9.16	118.86	1.41	5.29	0.215
24-hour DBP (mmHg)	68.94	1.32	7.45	68.74	2.06	8.75	69.20	1.52	5.68	0.865
24-hour MAP (mmHg)	86.22	1.15	6.5	86.54	1.88	7.99	85.81	1.10	4.13	0.758
Daytime BP										
SBP (mmHg)	126.18	1.53	8.66	128.79	2.26	9.60	122.81	1.62	6.06	0.051
DBP (mmHg)	71.96	1.54	8.73	72.47	2.34	9.93	71.30	1.93	7.20	0.714
MAP (mmHg)	89.86	1.31	7.39	91.01	2.04	8.65	88.38	1.42	5.31	0.326
Nighttime BP (n=30; Caucasians n=17; East Asians n=13)										
SBP (mmHg)	114.58	1.64	8.98	115.99	2.54	10.47	112.75	1.81	6.51	0.336
DBP (mmHg)	65.20	1.27	6.93	65.17	2.13	8.77	65.32	1.02	3.66	0.98
MAP (mmHg)	81.80	1.23	6.71	82.12	2.06	8.51	81.38	0.96	3.47	0.749
Body composition										

Baseline characteristics	All participants (n=32)			*Caucasians (n=18)			**East Asians (n=14)			P-value (Sig. 2-tailed)
	Mean	SEM	STD	Mean	SEM	STD	Mean	SEM	STD	
Grip strength mean (kg)	40.41	1.14	6.45	40.19	1.76	7.48	40.69	1.36	5.10	0.833
Grip strength max (kg)	42.77	1.18	6.68	42.43	1.82	7.71	43.21	1.42	5.33	0.749
Body fat (%)	16.37	0.91	5.16	17.09	1.28	5.42	15.44	1.29	4.84	0.379
Body fat (kg)	12.62	1.00	5.67	13.64	1.45	6.17	11.31	1.30	4.86	0.254
Lean (%)	83.36	1.19	5.56	82.57	1.62	5.60	84.31	1.79	5.66	0.478
Lean (kg)	61.88	1.14	6.47	63.40	1.46	6.18	59.92	1.75	6.53	0.134
Total (kg)	74.25	1.96	11.08	76.60	2.75	11.68	71.23	2.63	9.86	0.178
Dry lean weight (kg)	18.03	0.52	2.94	18.31	0.67	2.85	17.67	0.83	3.12	0.553
Water (%)	59.63	0.94	5.32	59.49	1.36	5.76	59.81	1.31	4.91	0.87
Water (lt)	43.85	0.76	4.27	45.09	1.01	4.27	42.25	1.03	3.85	0.061
Impedance 50KHz	490.13	18.48	104.54	463.94	28.97	122.91	523.79	17.19	64.31	0.109

*Caucasians: Normal BMI = 18.50 – 24.99 kg/m²; Underweight BMI: < 18.5 kg/m²; Overweight BMI: = 25.0 – 29.99 kg/m²; Obese BMI: > = 30.00 kg/m²

**Asians: Normal BMI: = 18.5 -22.99 kg/m²; Overweight BMI: = 23-27.49 kg/m²; Obese BMI: >= 27.5 kg/m²

5.6.2 Nutritional intakes of participants

The average energy intake of participants, recorded from 3-day dietary diaries by each participant, are presented in **Table 5.4**. **Table 5.4** showed that there is no statistically significant difference of nutritional intake of Caucasians and East Asians as p-value are all higher than 0.05. Reported energy intake (EI) was related to estimated basal metabolic rate (BMR) based on self-reported body weight and age. As reported in **Table 5.4**, EI:BMR ratios of both ethnicities (Caucasians: 1.23; East Asians: 1.15) are lower than 1.35, which is likely to reflect the presence of underreporting of energy intake (**Table 5.4**).

Table 5.4 Daily intakes of nutrients by Caucasians and East Asians from 3-d dietary records

Baseline characteristics	Caucasians					East Asians					P-value
	Mean	SEM	STD	Range	N	Mean	SEM	STD	Range	N	
Protein (g)	101.24	8.77	37.21	(55.3, 181.3)	18	93.05	6.47	24.20	(47.8, 127.7)	14	0.481
Fat (g)	81.40	7.31	31.01	(28.0, 152.3)	18	84.31	6.83	25.56	(40.2, 131.8)	14	0.778
Carbohydrate (g)	253.15	26.60	112.86	(132.3,562.5)	18	231.49	13.80	51.62	(156.9, 330.3)	14	0.476
Energy intake (EI) (kcal)	2148.98	148.00	627.92	(1194.6, 3934.1)	18	1966.20	90.16	337.33	(1214.9, 2635.3)	14	0.334
Energy intake (EI) (kJ)	8977.63	614.65	2607.75	(5051.8, 16553.6)	18	8346.21	401.51	1502.32	(5125.4, 11047.3)	14	0.427
Total Saturates (g)	28.84	3.02	12.80	(8.2, 61.1)	18	28.81	2.23	8.35	(12.9, 44.5)	14	0.995
Total monounsaturate (g)	29.09	3.36	14.25	(7.9, 59.8)	18	30.67	3.00	11.24	(13.4, 51.8)	14	0.736
Total polyunsaturate (g)	12.89	1.52	6.44	(4.0, 28.3)	18	16.70	2.05	7.67	(8.4, 32.5)	14	0.137
Cholesterol (mg)	389.83	53.12	225.39	(51.1, 776.8)	18	374.23	52.39	196.03	(127.6, 711.5)	14	0.839
Total trans fatty acid (g)	1.60	0.34	1.44	(0.4, 5.9)	18	1.47	0.29	1.08	(0.3, 4.4)	14	0.781
Total n-3 fatty acid (g)	0.94	0.48	2.04	(0.08, 8.75)	18	0.78	0.21	0.79	(0.03, 2.78)	14	0.789
Total n-6 fatty acid (g)	3.47	0.86	3.64	(0.37, 13.69)	18	4.78	1.09	4.09	(0.36, 14.86)	14	0.345
EI/BMR ratio	1.23	0.10	0.41	(0.75, 2.46)	18	1.15	0.06	0.24	(0.64, 1.48)	14	0.543
Sodium	2841.47	305.68	1296.91	(1186.80, 6565.71)	18	12597.16	9756.05	36503.78	(1352.75,139251.89)	14	0.336

Data are presented as mean ± STD, mean ± SEM or range (minimum, maximum);

An EI:BMR < 1.35 was considered to represent underreporting and an EI:BMR ≥2.4 as overreporting of EI;

Abbreviations: N, number of subject;

5.6.3. Anthropometric data of participants

The weight, BMI, handgrip strength and body composition of the participants, including body fat, water mass and impedance did not differ significantly between baseline, washout period and after consumption of extra virgin olive oil (EVOO) and butter (**Table 5.5**). These are suggestive of good compliance with instructions to not change lifestyle habits (diet, physical activity) during the intervention.

Table 5.5 Effects of extra virgin olive oil and butter on anthropometric data in total participants (n=32).

Variable	Unadjusted					Adjustment for baseline and washout					
	N	Olive Oil		Butter		P	Olive Oil		Butter		P**
		Mean	SEM	Mean	SEM		LSM	SEM	LSM	SEM	
Grip strength mean (kg)	32	41.46	1.29	40.43	1.25	0.20	41.46	0.77	40.43	0.50	0.20
Grip strength maximum (kg)	32	43.69	1.37	42.94	1.28	0.35	43.69	0.78	42.94	0.63	0.37
Body fat (%)	32	16.45	1.00	16.93	0.96	0.26	16.45	0.41	16.93	0.37	0.27
Body fat (kg)	32	12.79	1.08	13.30	1.08	0.20	12.79	0.36	13.30	0.33	0.21
Lean (kg)	32	61.95	1.22	62.31	1.22	0.36	61.95	0.30	62.31	0.35	0.36
Total (kg)	32	74.52	2.08	75.39	2.09	0.09	74.52	0.47	75.39	0.39	0.09
Dry lean weight (kg)	32	18.12	0.61	18.31	0.57	0.35	18.12	0.17	18.31	0.14	0.34
Water (%)	32	59.35	1.01	58.68	0.87	0.22	59.35	0.53	58.68	0.44	0.23
Water (lt)	32	43.77	0.76	43.67	0.67	0.78	43.67	0.23	43.77	0.31	0.37
Basal MET.RATE (kcal)	32	1904.22	32.21	1892.34	33.49	0.26	1904.22	8.83	1892.34	10.63	0.27
*BMR (kcal/kg)	32	25.43	0.35	25.66	0.36	0.06	25.43	0.17	25.66	0.17	0.07
EST.AVERAGE REQ: (kcal)	32	2772.31	107.11	2897.25	63.20	0.12	2772.31	79.74	2897.25	24.73	0.12
Impedance 50KHz	32	497.41	11.63	505.44	10.58	0.37	497.41	10.42	505.44	8.62	0.39

* Values BMR (kcal/kg) determined by the Henry's equation (Henry 2005).

5.6.4 Blood Pressure

5.6.4.1 Resting Blood Pressure and 24 - hour/Ambulatory Blood Pressure

Results analysed for the total sample showed no significant difference in resting blood pressure when comparing interventions; however, statistically significant differences on 24 hours SBP, daytime SBP, night-time DBP and night-time MAP were observed as shown in **Table 5.6**. Overall, after 30ml of EVOO consumption, the 24-hour systolic blood pressure was significantly reduced by 4.23 mmHg ($p = 0.02$) in unadjusted comparisons, 4.23 mmHg ($p^*=0.021$) with adjustment for baseline and washout period and 4.23 mmHg ($p^{**}=0.032$) with adjustment for baseline, washout, BMI, age, sodium intake and energy intake respectively (**Table 5.6**). Also, followed by EVOO consumption compared with butter in the group of all participants, the daytime SBP was significantly reduced by 5.13 mmHg ($p=0.008$), 5.13 mmHg ($p^*=0.01$) and 5.13 mmHg ($p^{**}=0.014$) respectively (**Table 5.6**). Finally, after EVOO consumption, the night-time DBP was significantly decreased by 3.81mmHg ($p = 0.023$), 3.98 mmHg ($p^* = 0.021$) and 3.98 mmHg ($p^{**} = 0.017$) respectively while the night-time MAP was significantly reduced by 3.73 mmHg ($p=0.019$), 3.82 mmHg ($p^*=0.024$) and 3.82 mmHg ($p^{**}=0.02$) respectively (**Table 5.6**).

Results comparing the effect of interventions only on Caucasian individuals are shown in **Table 5.7**. Finding indicated that night-time DBP and night-time MAP was significantly reduced by 4.436 mmHg ($P^{**}=0.042$) with adjustment for baseline, washout, BMI, age. Sodium and energy intake and 4.124 mmHg ($P^{**}=0.042$) with adjustment for baseline, washout, BMI, age. Sodium and energy intake respectively after EVOO consumption compared with butter intake.

Table 5.8 presents the results for only East Asians participants; the 24-hour systolic blood pressure was significantly reduced after EVOO by 5.91 mmHg ($p = 0.017$) without adjustment, 5.91 mmHg ($p^*= 0.015$) with adjustment for baseline and washout period and 5.91 mmHg ($p^{**} = 0.045$) with adjustment for baseline, washout, BMI, age, sodium and energy intake after extra virgin olive oil consumption respectively. Furthermore, daytime SBP was significantly reduced by 8.18 mmHg ($p= 0.004$), 8.18 mmHg ($P^{**} = 0.007$) and 8.18 mmHg ($P^{**} = 0.021$) after extra virgin olive oil consumption (**Table 5.8**). Daytime MAP was significantly reduced by 5.22 mmHg ($p=0.03$), 0.039 ($p^*=0.039$) and 0.039 ($p^{**}=0.032$) respectively after extra virgin olive oil intake too (**Table 5.8**).

Table 5.6 Effects of extra virgin olive oil and butter on blood pressure in Caucasians and East Asian healthy men of all participants (n=32).

Variable	Unadjusted					Adjustment for baseline and washout					Adjustment for baseline, washout, BMI, age, sodium intake and energy intake					
	N	Olive Oil		Butter		P	Olive Oil		Butter		p*	Olive Oil		Butter		p**
		Mean	SEM	Mean	SEM		LSM	SEM	LSM	SEM		LSM	SEM	LSM	SEM	
Resting SBP (mmHg)	32	116.866	1.797	118.525	1.810	0.358	116.866	1.566	118.525	1.517	0.361	116.87	1.64	118.53	1.41	0.335
Resting DBP (mmHg)	32	69.3344	1.613	68.366	1.650	0.535	69.334	1.191	68.366	1.153	0.543	68.217	1.724	68.394	0.953	0.937
Resting HR	32	72.447	2.144	72.616	1.924	0.935	72.447	1.739	72.616	1.232	0.937	69.706	2.056	72.922	1.373	0.190
24-Hour ABP	N	Olive Oil		Butter		P	Olive Oil		Butter		p*	Olive Oil		Butter		p**
		Mean	SEM	Mean	SEM		LSM	SEM	LSM	SEM		LSM	SEM	LSM	SEM	
SBP (mmHg)	32	117.81	1.47	122.04	1.52	0.020	117.81	1.196	122.04	1.344	0.021	117.81	1.28	122.04	1.42	0.032
DBP (mmHg)	32	68.519	1.324	70.553	1.296	0.179	68.519	1.046	70.553	1.017	0.123	68.519	1.008	70.553	1.041	0.114
MAP (mmHg)	32	85.469	1.181	87.863	1.221	0.086	85.469	0.875	87.863	1.036	0.056	85.469	0.905	87.863	1.075	0.064
Daytime ABP	N	Olive Oil		Butter		P	Olive Oil		Butter		p*	Olive Oil		Butter		p**
		Mean	SEM	Mean	SEM		LSM	SEM	LSM	SEM		LSM	SEM	LSM	SEM	
SBP (mmHg)	32	123.20	1.693	128.33	1.433	0.008	123.20	1.411	128.33	1.086	0.01	123.20	1.48	128.33	1.12	0.014
DBP (mmHg)	32	72.637	1.527	74.184	1.375	0.388	72.638	1.299	74.184	1.025	0.346	72.673	1.304	74.184	1.034	0.350
MAP (mmHg)	32	90.006	1.444	92.241	1.165	0.170	90.006	1.234	92.241	0.910	0.155	90.006	1.258	92.241	0.928	0.168
Nighttime ABP	N	Olive Oil		Butter		P	Olive Oil		Butter		p**	Olive Oil		Butter		p***
		Mean	SEM	Mean	SEM		LSM	SEM	LSM	SEM		LSM	SEM	LSM	SEM	
SBP (mmHg)	32	111.452	1.494	115.084	1.711	0.057	111.680	1.230	115.353	1.685	0.068	111.68	1.187	115.353	1.749	0.068
DBP (mmHg)	32	62.98	1.317	66.79	1.505	0.023	62.997	1.108	66.98	1.362	0.021	63.00	0.991	66.98	1.46	0.017
MAP (mmHg)	32	79.57	1.149	83.30	1.509	0.019	79.65	0.955	83.470	1.405	0.024	79.65	0.91	83.470	1.49	0.02

* Estimated from repeated measures ANOVA of the Olive Oil and Butter with baseline and washout value as covariate.

**Estimated from repeated measures ANOVA of the Olive Oil and Butter with baseline, washout, BMI, age, sodium intake and energy intake value as covariate.

LSM = Least Squared Means (Marginal means).

Table 5.7 Effects of Extra virgin olive oil and Butter on blood pressure in Caucasians participants (n=18).

Variable	N	Unadjusted					Adjustment for baseline and washout					Adjustment for baseline, washout, BMI, age, sodium intake and energy intake				
		Olive Oil		Butter		P	Olive Oil		Butter		P*	Olive Oil		Butter		p**
		Mean	SEM	Mean	SEM		LSM	SEM	LSM	SEM		LSM	SEM	LSM	SEM	
Resting SBP (mmHg)	18	118.10	2.29	119.13	2.56	0.712	118.10	2.14	119.13	2.02	0.704	120.211	1.469	123.139	2.072	0.302
Resting DBP (mmHg)	18	68.22	2.08	68.39	2.29	0.933	68.22	1.59	68.39	1.19	0.934	68.378	1.337	69.761	0.989	0.346
Resting HR	18	69.71	2.33	72.92	2.86	0.226	69.71	1.81	72.92	1.54	0.201	69.706	2.056	72.922	1.373	0.19
24-Hour ABP																
SBP (mmHg)	18	120.21	2.05	123.14	2.07	0.269	120.21	1.36	123.14	1.98	0.263	120.21	1.49	123.14	2.11	0.322
DBP (mmHg)	18	68.38	1.96	69.76	1.56	0.489	68.38	1.41	69.76	1.08	0.418	68.378	1.337	69.761	0.989	0.346
MAP (mmHg)	18	86.26	1.82	87.52	1.47	0.508	86.26	1.13	87.52	1.23	0.418	86.256	1.019	87.522	1.032	0.384
Daytime ABP																
SBP (mmHg)	18	127.23	1.98	129.98	1.93	0.3	127.23	1.81	129.98	1.67	0.329	127.23	1.86	129.98	1.83	0.352
DBP (mmHg)	18	73.79	2.14	73.66	1.72	0.956	73.79	1.56	73.66	1.13	0.948	73.789	1.616	73.656	0.889	0.948
MAP (mmHg)	18	92.22	2.03	92.13	1.38	0.97	92.22	1.62	92.13	0.98	0.967	92.22	1.56	92.13	0.83	0.967
Nighttime ABP																
SBP (mmHg)	18	112.37	2.20	116.59	2.22	0.147	112.82	1.23	117.16	2.33	0.148	112.824	1.418	117.159	2.045	0.139
DBP (mmHg)	18	62.33	2.05	66.42	1.92	0.078	62.29	1.63	66.72	1.79	0.082	62.288	1.416	66.724	1.413	0.042
MAP (mmHg)	18	79.47	1.80	83.40	1.96	0.079	79.59	1.30	83.71	1.83	0.101	79.588	1.177	83.712	1.236	0.042
<i>*Estimated from repeated measures ANOVA of the Olive Oil and Butter with baseline and washout value as covariate.</i>																
<i>**Estimated from repeated measures ANOVA of the Olive Oil and Butter with baseline, washout, BMI, age, sodium intake and energy intake value as covariate.</i>																
LSM = Least Squared Means (Marginal means).																

Table 5.8 Effects of extra virgin olive oil and Butter on blood pressure in East Asians participants (n=14).

East Asians		Unadjusted					Adjustment for baseline and washout					Adjustment for baseline, washout, BMI, age, sodium intake and energy intake				
Variable	N	Olive Oil		Butter		P	Olive Oil		Butter		P*	Olive Oil		Butter		p**
		Mean	SEM	Mean	SEM		LSM	SEM	LSM	SEM		LSM	SEM	LSM	SEM	
Resting SBP (mmHg)	14	115.28	0.91	117.75	2.60	0.264	115.28	2.51	117.75	2.41	0.298	114.721	2.245	120.629	1.778	0.047
Resting DBP (mmHg)	14	70.77	2.57	68.33	2.45	0.308	70.77	1.95	68.33	2.24	0.333	68.700	1.764	71.571	2.103	0.197
Resting HR	14	75.97	3.77	72.22	2.54	0.260	75.97	2.98	72.22	1.91	0.283	75.971	2.142	72.221	2.163	0.296
24-Hour ABP																
SBP (mmHg)	14	114.72	1.84	120.63	2.27	0.017	114.72	1.80	120.63	1.58	0.015	114.72	2.22	120.63	1.62	0.045
DBP (mmHg)	14	68.70	1.77	71.57	2.22	0.239	68.7	1.68	71.57	1.91	0.22	68.700	1.764	71.571	2.103	0.197
MAP (mmHg)	14	84.46	1.37	88.30	2.11	0.067	84.46	1.33	88.30	1.80	0.072	84.457	1.601	88.300	1.847	0.076
Daytime ABP																
SBP (mmHg)	14	118.02	2.32	126.20	2.07	0.004	118.02	2.07	126.20	1.14	0.007	118.02	2.59	126.20	0.96	0.021
DBP (mmHg)	14	71.16	2.18	74.86	2.30	0.175	71.16	2.15	74.86	1.93	0.195	71.157	2.296	74.864	1.910	0.095
MAP (mmHg)	14	87.16	1.83	92.38	2.05	0.030	87.16	1.80	92.38	1.67	0.039	87.16	2.14	92.38	1.49	0.032
Nighttime ABP																
SBP (mmHg)	14	110.19	1.90	112.99	2.67	0.224	110.19	1.43	112.99	1.82	0.178	110.185	1.766	112.992	2.146	0.246
DBP (mmHg)	14	63.92	1.42	67.31	2.50	0.179	63.92	1.51	67.31	1.88	0.122	63.923	1.385	67.308	1.609	0.081
MAP (mmHg)	14	79.72	1.23	83.15	2.45	0.142	79.72	1.15	83.15	1.88	0.105	79.723	1.321	83.154	1.982	0.129
<i>*Estimated from repeated measures ANOVA of the Olive Oil and Butter with baseline and washout value as covariate.</i>																
<i>**Estimated from repeated measures ANOVA of the Olive Oil and Butter with baseline, washout, BMI, age, sodium intake and energy intake value as covariate.</i>																
LSM = Least Squared Means (Marginal means).																

5.6.4.2 Blood lipid profile & Novel Circulating biomarkers

Results analysed for the total sample showed no significant differences in most blood lipid profile as well as inflammatory biomarkers when comparing interventions; however, statistically significant differences between East Asians and Caucasians for sVCAM-1 ($p=0.003$) were observed (**Table 5.9**). After EVOO, TC was significantly reduced by 0.216 ($p=0.005$) with unadjustment, 0.209 ($p^*=0.008$) with adjustment for baseline and washout, and 0.209 ($p^{**}=0.011$) with adjustment for baseline, washout, BMI, age, energy intake, saturated fatty acids and cholesterol respectively in the group of all participants after extra virgin olive oil intake (**Table 5.10**). LDL-C was significantly reduced by 0.18 mmol/L ($p = 0.011$), 0.18 mmol/L ($p^* = 0.012$) and 0.18 mmol/L ($p^{**} = 0.016$) after EVOO respectively in the group of all participants (**Table 5.10**).

In Caucasians, EVOO significantly reduced LDL-C by 0.217 mmol/L ($p=0.039$) without adjustment, 0.217 mmol/L ($p^* = 0.046$) with adjustment for baseline and washout and 0.217 mmol/L ($p^{**} = 0.048$) with adjustment for baseline, washout, BMI, age, energy intake, saturated fatty acids and cholesterol (**Table 5.11**). In the group of East Asians (**Table 5.12**), TC was significantly decreased by 0.207 mmol/L ($p=0.03$). However, the significance remained robust after both adjustments as covariates. Among the East Asian participants, E-selectin was significantly increased by 17.21 ng/mL ($p=0.004$) and 17.21 ng/mL ($p^{***}=0.012$) (**Table 5.12.1**).

Table 5.9 Participants baseline characteristic of blood-borne biomarkers.

Baseline characteristics	Caucasians			East Asians			P-value
	Mean	SEM	N	Mean	SEM	N	
TC (mmol/L)	4.23	0.25	18	4.49	0.19	13	0.449
HDL-C (mmol/L)	1.34	0.08	18	1.32	0.11	13	0.859
LDL-C (mmol/L)	2.48	0.24	18	2.72	0.16	13	0.445
Triglycerides (mmol/L)	0.89	0.09	18	0.90	0.09	14	0.965

Caucasians including British, Italian, Croatian, Irish, American; East Asians including Chinese, Hong Kong China, Malaysians.

Lipid profile including TC, HDL-C, LDL-C and TG analysed in this table was collected from the Laboratories of the Integrated Laboratory Medicine in Freeman Hospital at Newcastle upon Tyne, UK.

Table 5.10 Effects of Extra Virgin Olive oil and butter on blood-borne biomarkers in all Caucasians and East Asian male participants (n=32).

Variable	N	Unadjusted					Adjustment for baseline and washout					Adjustment for baseline, washout, BMI, age, energy intake, saturated fatty acids and cholesterol				
		Olive oil		Butter		P	Olive oil		Butter		P*	Olive oil		Butter		P**
		Mean	SEM	Mean	SEM		LSM	SEM	LSM	SEM		LSM	SEM	SEM	SEM	
TC (mmol/L)	31	4.28	0.15	4.49	0.15	0.005	4.23	0.06	4.44	0.06	0.008	4.226	0.052	4.435	0.063	0.011
HDL (mmol/L)	31	1.30	0.66	1.34	0.07	0.172	1.33	0.02	1.36	0.03	0.274	1.329	0.016	1.358	0.024	0.277
LDL (mmol/L)	31	2.48	0.12	2.66	0.13	0.011	2.48	0.04	2.66	0.06	0.012	2.477	0.035	2.657	0.063	0.016
Triglycerides (mmol/L)	32	1.35	0.30	1.13	0.10	0.371	1.35	0.29	1.13	0.10	0.331	1.347	0.292	1.128	0.088	0.349
IL-6 (pg/mL)	32	292.75	3.69	289.46	2.92	0.543	290.71	7.89	295.71	2.95	0.581	290.71	11.70	295.71	1.76	0.733
sICAM-1 (ng/mL)	32	318.58	17.36	347.41	22.05	0.298	318.58	28.55	356.02	32.49	0.466	318.58	34.10	356.02	20.87	0.391
sVCAM-1 (ng/mL)	32	101.13	5.57	98.70	8.14	0.764	104.83	12.57	109.63	16.40	0.788	104.83	15.86	109.63	15.51	0.787
P-selectin (ng/mL)	32	613.14	67.58	525.93	40.84	0.318	498.90	66.78	570.76	71.13	0.448	498.90	74.10	570.76	92.20	0.376
E-selectin (ng/mL)	32	58.50	9.03	57.98	8.83	0.936	55.19	4.03	50.92	5.14	0.211	55187.27	5646.62	50915.46	6671.76	0.084
Syndecan-1 (pg/mL)	32	20.02	4.52	19.721	4.334	0.92	29.29	7.58	31.09	1.90	0.823	29287.00	7299.82	31085.70	1290.84	0.786
PAI-1 (ng/mL)	32	148.36	13.26	151.79	12.74	0.855	146.52	21.54	160.42	19.55	0.698	146.52	29.71	160.42	23.75	0.767
CRP (pg/mL)	32	36.23	4.12	36.50	2.508	0.958	40.27	10.59	32.684	5.121	0.166	402733.30	428499.29	326844.40	351692.8.21	0.135

*Estimated from repeated measures ANOVA of olive oil with baseline value as covariate.

**Estimated from repeated measures ANOVA of olive oil with baseline, washout, BMI, age, energy intake, saturated fatty acids and dietary cholesterol value as covariate.

LSM = Least Squared Means (Marginal means).

Table 5.11 Effects of Extra Virgin Olive oil on blood-borne biomarkers in Caucasians male participants (n=18).

Variable	N	Unadjusted					Adjustment for baseline and washout					Adjustment for baseline, washout, BMI, age, energy intake, saturated fatty acids and cholesterol				
		Olive oil		Butter		P	Olive oil		Butter		P*	Olive oil		Butter		P**
		Mean	SEM	Mean	SEM		LSM	SEM	LSM	SEM		LSM	SEM	LSM	SEM	
TC (mmol/L)	18	4.13	0.20	4.36	0.21	0.059	4.13	0.08	4.36	0.07	0.056	4.133	0.065	4.356	0.076	0.082
HDL (mmol/L)	18	1.36	0.08	1.37	0.08	0.767	1.36	0.02	1.38	0.04	0.764	1.356	0.025	1.367	0.039	0.782
LDL (mmol/L)	18	2.47	0.18	2.69	0.19	0.039	2.47	0.06	2.69	0.07	0.048	2.471	0.046	2.688	0.076	0.072
Non-HDL (mmol/L)	18	2.78	0.22	3.04	0.23	0.037	2.78	0.08	3.04	0.09	0.042	2.778	0.06	3.039	0.103	0.065
Triglycerides (mmol/L)	18	0.92	0.10	0.93	0.08	0.958	0.92	0.10	0.93	0.06	0.959	0.922	0.075	0.928	0.055	0.946

**Estimated from repeated measures ANOVA of olive oil and butter with baseline and washout value as covariate.*

***Estimated from repeated measures ANOVA of olive oil and butter with baseline, washout, BMI, age, energy intake, saturated fatty acids and cholesterol value as covariate.*

Table 5.11.1 Effects of Extra Virgin Olive oil on blood-borne biomarkers in Caucasians male participants (n=18).

Variable	N	Unadjusted				P	Adjustment for washout, BMI, age, energy intake, saturated fatty acids and cholesterol				p***
		Olive oil		Butter			Olive oil		Butter		
		Mean	SEM	Mean	SEM		LSM	SEM	LSM	SEM	
IL-6 (pg/mL)	18	296.40	3.27	291.83	3.30	0.338	296.40	2.584	291.83	3.039	0.348
PAI-1 (ng/mL)	18	153.47	17.85	145.88	14.82	0.763	153.47	20.18	145.87	15.30	0.779
sICAM-1 (ng/mL)	18	325.74	24.63	384.12	32.96	0.142	325.74	25.14	384.12	26.50	0.131
sVCAM-1 (ng/mL)	18	102.31	8.30	107.29	10.90	0.654	102.31	8.48	107.29	8.407	0.596
P-selectin (ng/mL)	18	593.25	49.24	567.97	42.73	0.67	593.24	50.07	567.97	42.05	0.667
E-selectin (ng/mL)	18	73.860	12.186	59.552	11.355	0.159	73.86	12.382	59.552	11.73	0.205
Syndecan-1 (pg/mL)	18	14.088	5.704	13.484	5.146	0.678	14.088	5.328	1.3484	50.80	0.669
CRP (pg/mL)	18	34.901	7.017	36.879	3.530	0.815	34.901	6.677	36.879	27.43	0.773

**Estimated from repeated measures ANOVA of olive oil and butter with baseline and washout value as covariate.*

***Estimated from repeated measures ANOVA of olive oil and butter with baseline, washout, BMI, age, energy intake, saturated fatty acids and cholesterol value as covariate.*

**** Estimated from repeated measures ANOVA of olive oil and butter with washout, BMI, age, energy intake, saturated fatty acids and cholesterol value as covariate.*

LSM = Least Squared Means (Marginal means).

Table 5.12. Effects of extra virgin olive oil and butter on blood-borne biomarkers in men of East Asians (n=14).

Variable	N	Unadjusted					Adjustment for baseline and washout					Adjustment for baseline, washout, BMI, age, energy intake, saturated fatty acids and cholesterol				
		Olive oil		Butter		P	Olive oil		Butter		P*	Olive oil		Butter		P**
		Mean	SEM	Mean	SEM		LSM	SEM	LSM	SEM		LSM	SEM	LSM	SEM	
TC (mmol/L)	14	4.46	0.21	4.66	0.22	0.03	4.35	0.08	4.55	0.10	0.079	4.354	0.065	4.546	0.111	0.113
HDL (mmol/L)	14	1.24	0.11	1.31	0.12	0.086	1.29	0.03	1.35	0.04	0.161	1.292	0.017	1.346	0.026	0.136
LDL (mmol/L)	13	2.49	0.15	2.62	0.19	0.16	2.49	0.07	2.62	0.10	0.181	2.485	0.044	2.615	0.103	0.323
Non-HDL (mmol/L)	13	3.29	0.24	3.36	0.24	0.561	3.13	0.13	3.20	0.09	0.632	3.131	0.105	3.200	0.108	0.65
Triglycerides (mmol/L)	14	1.89	0.65	1.39	0.18	0.361	1.89	0.57	1.39	0.16	0.271	1.893	0.508	1.386	0.128	0.276

**Estimated from repeated measures ANOVA of olive oil and butter with baseline and washout value as covariate.*

***Estimated from repeated measures ANOVA of olive oil and butter with baseline, washout, BMI, age, energy intake, saturated fatty acids and cholesterol value as covariate.*

Table 5.12.1 Effects of extra virgin olive oil and butter on blood-borne biomarkers in men of East Asians (n=14).

Variable	N	Unadjusted					Adjustment for washout, BMI, age, energy intake, saturated fatty acids and cholesterol				
		Olive oil		Butter		P	Olive oil		Butter		p***
		Mean	SEM	Mean	SEM		LSM	SEM	LSM	SEM	
IL-6 (pg/mL)	14	288.06	7.30	286.41	5.18	0.882	288.06	7.883	286.414	5.107	0.891
Human PAI-1 (ng/mL)	14	141.78	20.39	159.39	22.53	0.549	141.783	21.020	159.389	21.903	0.573
sICAM-1 (ng/mL)	14	309.37	24.71	300.21	22.77	0.812	309.366	25.475	300.205	25.225	0.815
sVCAM-1 (ng/mL)	14	99.62	7.29	87.66	12.03	0.33	99.621	7.026	87.656	13.344	0.403
P-selectin (ng/mL)	14	638.72	143.98	471.88	74.97	0.38	638.717	166.474	471.88	82.988	0.443
E-selectin (ng/mL)	14	38.75	11.89	55.96	14.41	0.004	38.75	9.707	55.96	11.763	0.012
Syndecan-1 (pg/mL)	14	27.6520	6.9609	27.7397	7.0036	0.99	27.652	62.595	27.740	74.180	0.989
CRP (pg/mL)	14	37.9361	3.0298	36.002	3.647	0.671	37.938	27.723	36.002	30.856	0.692

*** Estimated from repeated measures ANOVA of olive oil and butter with washout, BMI, age, energy intake, saturated fatty acids and cholesterol value as covariate.
LSM = Least Squared Means (Marginal means).

5.7. Discussion

Despite the recent proliferation of research on the health benefits of olive oil, few trials have been carried out in Asian individuals. To the best of our knowledge, this is the first trial assessing the impact of olive oil comparing two ethnic groups. Data from this study will substantiate our evidence on the efficacy of olive oil in improving cardiovascular risk biomarkers.

5.7.1 Principal findings

In the present study the benefits of dietary supplementation with extra virgin olive oil (EVOO) on serum inflammatory markers and lipid profile were explored, in a sample of healthy adults of two ethnicities. 30ml of EVOO effects were tested against 30g of butter and the results are significant difference between the two intervention periods in all participants.

The present study found that the consumption of 30 ml of EVOO over a 2-week period produced a positive effect on 24-hour SBP including daytime SBP, night-time DBP and MAP and TC, LDL-C for all participants. For East Asians participants, olive oil consumption exerts a beneficial effect on 24-hour SBP and daytime SBP, MAP while night-time DBP was improved among Caucasians after EVOO intake. EVOO intake also has a positive effect on blood lipids such as TC and circulating biomarkers such as E-selectin in East Asians while LDL-C and non-HDL-C are improved among Caucasians after EVOO intake.

Overall, night-time BP (night-time DBP, night-time MAP) were shown to be improved by Caucasians while day-time BP (daytime SBP, daytime MAP) were shown to be improved by East Asians after EVOO consumption. However, 24-hour BP were significantly improved in East Asians while no changes in 24-hour BP in Caucasians. This shows that EVOO intervention presents a better overall improvement on BP in East Asians than Caucasians.

East Asians shows a significant improvement on E-selectin after EVOO intake while total-cholesterol is improved among East Asians and LDL-cholesterol is improved among Caucasians. East Asians show a better outcome in terms of cardiovascular biomarkers.

These results indicate that consuming EVOO as part of an overall diet brings benefits that in the long term may translate into reducing the risk of CVDs. These results are significant given the fact that high BP remains ranked 1st among the leading 30 level-3 global risk

factors for DALYs (Zhou, Wang et al. 2019). Importantly, these effects were unrelated to differences and changes in body fats and BMI. These results support the recommendation of EVOO to decrease some cardiovascular risk factors in both Caucasians and East Asians; this is particularly important for East Asian individuals and populations whose cardiovascular risk may be present at lower BMIs (WHO Expert Consultation 2004).

5.7.2 Meaning of the current findings

The results of this dietary intervention indicated that 30ml intake of extra virgin olive oil (EVOO) daily with two weeks was associated with leading a significant improvement in 24-hour blood pressure for both Caucasians and East Asians in general. While night-time DBP as well as night-time MAP were achieved significant improvement among Caucasians male participants, daytime SBP and daytime MAP were gained significant improvement among East Asian male participants. In addition, sE-selectin was significantly improved among only East Asian participants after EVOO consumption. Therefore, if extra virgin olive oil (EVOO) is included in habitual diet regularly, it could help to prevent BP related conditions and in the long term reduce the levels of CV mortality and morbidity.

5.7.2.1 Possible mechanisms and implications for clinicians or policymakers.

The cardiovascular beneficial effects of EVOO are mainly attributed to its high content on MUFAs but also minor phenolic compounds act as antioxidants in EVOO, with the phenols oleuropein and hydroxytyrosol (HT) standing out nutritionally, HT represents one of the main polyphenol contents of EVOO and has anti-inflammatory and anti-teratogenic activity, improving the lipid profile, reducing oxidative stress, and activating inflammatory cells (Marcelino, Hiane et al. 2019).

Consuming EVOO increases the postprandial concentration of phenolic compounds in the plasma and in LDL-C and hydroxytyrosol and oleuropein, dose-dependently inhibit LDL oxidation *in vitro* and *in vivo*, repress superoxide-driven reactions, and break the chain-like propagation of lipid peroxides (Berrougui, Ikhlef et al. 2015). A study (Gimeno, Fitó et al. 2002) indicated that olive oil phenols are able to bind to LDL-C *in vitro* which are significantly associated with the delay in LDL oxidation. In this report it was suggested that olive oil phenols protect other phenols bound to LDL from oxidation. Similar results for mechanism

of action showed that the lower LDL oxidation rate, as well as the reduced uptake of oxidized LDL by macrophages after olive oil, may be due to the high concentration of both phenolic compounds as well as other unsaponifiable compounds present in extra-virgin olive oil. Furthermore, flavonoids and isoflavonoids as well as other linear isoprenoids exhibiting in vitro antioxidant activity could help limit LDL peroxidation (Ramirez-Tortosa, Urbano et al. 1999).

The mechanism of absorption of olive oil phenols is different and inadequate research among different ethnicities, let alone different polarity of oleuropein-glycoside, oleuropein- and ligstroside-aglycones, and tyrosol and hydroxytyrosol probably results in different mechanisms of absorption in different ethnicities (Maud et al., 2002). Furthermore, a study (Siefer et al, 2018) investigating the lipid-lowering effect of 30 mg/day hydroxytyrosol from different sources (a pure, synthetic product and an olive extract) in comparison to placebo in a healthy population showed that neither total cholesterol levels nor HDL-cholesterol levels were significantly affected by the absorption of hydroxytyrosol, yet, a significant LDL-cholesterol reduction was observed after intervention with the pure, synthetic hydroxytyrosol in comparison to placebo ($p = 0.0003$). This could potentially explain the outcome that a decrease in LDL-cholesterol in Caucasians was observed.

Antihypertensive mechanisms might be proposed for the fat component oleic acid (OA), which is the main fatty acid present in EVOO and minor constituents, most of which feature marked antioxidant properties. The MUFAs OA induced a marked and significant decrease in systolic BP, and the dose of MUFAs administered was closely correlated with the reduction in BP (Terés, Barceló-Coblijn et al. 2008). Evidence showed that the potential mechanisms for EVOO to positively impact on hypertension is via bioactive minor components, mainly antioxidant polyphenolic components (oleuropein, hydroxytyrosol) (Duansak and Schmid-Schönbein 2013). Investigated antihypertensive mechanisms of action range across anti-oxidative activities, changes of aminopeptidase activities favouring the production of Ang 2-10 and the clearance of Ang III and Ang IV increase in the endothelial nitric oxide synthase (eNOS) expression, and reduction of plasma Ang II (Massaro, Scoditti et al. 2020). In addition, phenolic compounds, including the secoiridoids oleocanthal and oleacein in EVOO, help to stabilize atherosclerotic plaques in hypertensive patients by modulating the expression of mitochondrial membrane potential (MMPs) (Filipek, Czerwińska et al. 2017).

The expression of cell adhesion molecules, such as E-selectin are crucial for endothelial activation and regulate the inflammatory process. E-selectin was significantly reduced as consumption of high MUFAs (65.1%) rich in EVOO (Vafeiadou, Weech et al. 2015). It suggests a positive correlations between circulating E-selectin and blood pressure and the reduction in E-selectin may have contributed to the observed decrease in night SBP on human (Rao, Gurumurthy et al. 2011).

Overall, the excellent nutritional composition of EVOO, which includes a high content of monounsaturated fatty acids (oleic fatty acid) and minor phenolic compounds such as polyphenols (oleuropein and hydroxytyrosol) benefits risk factors on cardiovascular health. Despite so many positive effects of EVOO, still not enough is known about the mechanisms involved in these processes, especially the isolated actions of the components as well as the possible synergistic effects or antagonistic interactions with other components of the diet or medicine. Study (Osman et al, 2017) showed that the usage of EVOO with the therapeutic dose of ibuprofen presented synergistic effect in controlling the cardinal signs of acute inflammation rather than using nonsteroidal anti-inflammatory medication alone. Study (Natarajan et al., 2019) also indicated that tomatoes contain carotenoids which are fat soluble, and hence, absorption is increased with a fat medium such as EVOO.

From a nutrient perspective, this has been known for some time, e.g., with enhanced absorption of nonheme iron in the presence of vitamin C, and competitive inhibition of zinc absorption by iron (Maria and Hajo, 2020). From the perspective of dietary patterns, however, focusing on isolated nutrients cannot account for all interactions, and may result in erroneous conclusions. Important associations may be missed, or effects may be assumed in which none exist (Tapsell et al, 2016).

5.7.2.2. Comparison to previous studies

The current study results did not support the previous finding reported in **Chapter 4**, but DBP and TC are likely to be improved by olive oil intake. However, there is no significant reduction on TC, LDL and BP.

However, previous research supports the beneficial effect of olive oil on different vascular risk factors. A acute cross-over study on healthy men and women (Violi, Loffredo et al. 2015), the consumption of the Mediterranean meal supplemented with either 10 g of olive

or 10 g of corn oil reported significant ($P=0,014$) reductions of LDL-C after 2-hours of consumption; The changes in LDL-C before and after these meals were (olive oil before meal 67.9 ± 15.0 mg/dL and after 73.0 ± 18.0 mg/dL, $P<0.05$; corn oil before meal 68.6 ± 19.2 and after meal 90.5 ± 13.1 , $P=0.001$). In addition, significant improvements on LDL ($P<0.001$) but no significant changes were reported on triglycerides or HDL-C.

In agreement with the results in this study, other evidence suggested that diastolic blood pressure decreases after the olive oil consumption, -0.73 mm Hg, 95% CI $(-1.07, -0.40)$; $p < 0.001$, $I^2 = 86.9\%$, with high heterogeneity among the included studies. This reduction was mainly due to EVOO from 10 ml to 50 ml/day: -1.44 mm Hg, 95% CI $(-1.89, -1.00)$; $p < 0.001$ (Zamora-Zamora, Martínez-Galiano et al. 2018). Similarly, a 2-month crossover human trial in young women with mild hypertension reported that the consumption of a diet containing polyphenol-rich olive oil can decrease BP and lead to a significant ($P < 0.01$) decrease of 7.91 mm Hg in systolic and 6.65 mm Hg of diastolic BP compared with the baseline value (Moreno-Luna, Muñoz-Hernandez et al. 2012).

The regular consumption of EVOO (25 ml/day) by healthy individuals has been found to reduce the total cholesterol ($n=13$; SMD: -4.7 ; $p<0.05$), systolic blood pressure ($n=13$; SMD: -5 ; $P<0.05$) and diastolic blood pressure ($n=13$; SMD: -6 ; $P<0.05$), by means of the negative regulation in the ACE (angiotensin I) and NR1H2 genes (Martín-Peláez, Castañer et al. 2017).

Another systematic review has also reported medium effects after high phenolic olive oil for lowering systolic blood pressure ($n = 69$; SMD -0.52 ; CI $-0.77/-0.27$; $p < 0.01$ (Hohmann, Cramer et al. 2015). Nevertheless, in disagreement of the present clinical study, no effects were found for diastolic blood pressure ($n = 69$; SMD -0.20 ; CI $-1.01/0.62$; $p = 0.64$); TC ($n = 400$; SMD -0.05 ; CI: $-0.16/0.05$; $p = 0.33$); LDL-C ($n = 400$; SMD -0.03 ; CI: $-0.15/0.09$; $p = 0.61$) (Hohmann, Cramer et al. 2015). A recent randomized trial in middle-aged Chinese individuals also showed that olive oil consumption had not significantly effect on serum total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), or triacylglycerol (TG) (Sun, Xia et al. 2018). This study (Sun, Xia et al. 2018) was conducted in Yixing city, China, with participants for both gender, which was potentially the reason why there was no significant effect.

The inflammatory process is regulated by the expression of cell adhesion molecules, such as sE-selectin, which in this case is increased, and the consumption of EVOO, which has also been shown to be a factor responsible for the diminished sE-selectin when compared with the consumption of high-cholesterol diet. Such reduction might be also related as much to the presence of oleic fatty acid as to polyphenols (Katsarou, Kaliora et al. 2015).

Olive oil consumption shows a greater impact on people who are at high risk of cardiovascular factors, people experience vascular diseases and any cardiovascular risk factor than on healthy participants.

During 24 years of follow-up, among 10,240 incident cases of CVD, including 6,270 CHD cases and 3,970 stroke cases. After adjusting for major diet and lifestyle factors, compared with non-consumers, those with higher olive oil intake (>1/2 tablespoon/d or > 7g/d) had 15% lower risk of total CVD [pooled hazard ratio (95% confidence interval): 0.85 (0.77, 0.93)] and 21% lower risk of CHD [pooled hazard ratio (95% CI): 0.79 (0.70, 0.89)]. It estimated that replacing 5g of margarine, butter, mayonnaise, or dairy fat with the equivalent amount of olive oil was associated with 5-7% lower risk of total CVD and CHD (Guasch et al, 2020). Subjects undergoing coronary angiography experienced a significant decrease in plasma LDL-cholesterol (-9.52 ± 20.44 mg/dL, $p = .007$), a significant reduction in plasma CRP (-0.40 ± 0.52 mg/L, $p = .01$) after 25ml EVOO consumption (Khandouzi et al. 2021). Individuals at high cardiovascular risk in EVOO consumption had 39% (HR: 0.61; 95% CI: 0.44 to 0.85) CVD risk reduction, for each 10 g/d increase in EVOO consumption, CVD and mortality risk decreased by 10% and 7%, respectively (Guasch-Ferré et al., 2014).

In healthy male subjects, increase in high-density lipoprotein cholesterol levels was observed for low-, medium-, and high-polyphenol olive oil: mean change, 0.025 mmol/L (95% CI, 0.003 to 0.05 mmol/L), 0.032 mmol/L (CI, 0.005 to 0.05 mmol/L), and 0.045 mmol/L (CI, 0.02 to 0.06 mmol/L), respectively. Triglyceride levels decreased by an average of 0.05 mmol/L for all olive oils. Oxidative stress markers decreased linearly with increasing phenolic content. (Covas et al., 2006). In healthy English men and women subjects, LDL-C concentrations were significantly increased on olive oil (+0.38, 95% CI 0.16 to 0.60 mmol/L, $P < 0.0001$), Study showed that high phenolic extra virgin OO slightly reduce LDL-cholesterol compared to low phenolic (extra) virgin OO (mean difference [MD]: -0.14 mmol/L, 95%–CI: $-0.28, -0.01$) in healthy subjects (Schwingshackl et al., 2019).

5.7.2.3 Strengths

This study has a number of strengths. Firstly, the improvements observed in BP reported in this study were documented using robust methodology. BP changes were documented using ambulatory 24-hour BP rather than resting BP (more likely to be affected by external factors to the intervention). Secondly, self-reported daily checklist of EVOO showed a good acceptability and compliance with both EVOO and butter intake. No side-effect of interventions was reported. The fact that BMI, body composition and handgrip strength remained unchanged during the whole clinical trial, supports the presence of good compliance with maintaining usual diet and physical activity patterns. Another strength of the present study is its crossover design, which permitted the same participants to receive all olive oils and thereby minimized interferences with confounding variables. Furthermore, the dose of EVOO to be consumed in this study was accurately guided and explained by the researcher and provided as single bottle with graduated spoon, and the fact that the Filippo Berio Organic Extra Virgin Olive Oil (500ml) was of the same brand thereby reducing the risk of variability in consumption and composition of the olive oil. In addition, the measurements were all taken by the same researcher, so it would be consistent for every visit and reduce the possibility of bias.

In addition, this study also does not focus on single nutrients but EVOO food itself when it replaces participants' lunch meals. Assessing one single nutrient have had negative consequences as it often fails to consider substitution effects and associated foods. There is no effect of a nutrient in an absolute sense because this may change based on the replacement nutrient and the foods that deliver them.

The last advantage that only men were studied, excludes the confounding associated with hormonal changes during menstrual cycles affecting outcome measures in women. Based on the outcome of survey chapter, which constitute mostly female, thus male becomes the main target in this trial study. Males are not subject to frequent hormonal changes. Fluctuating hormones and differences between male and female study subjects could all complicate the design of the study and the interpretation of the results. For instance, female participants would be asked to postpone or be extend their EVOO/butter intervention or washout period for at least one week if female participants start their menstrual cycle in the middle of the trial due to irregular menstrual cycle. In addition, 30ml extra virgin olive oil daily intake, containing vitamin E, helps to promote the formation of estrogen in female body.

Evidence showed that hormones changes and endocrine disorders could alter plasma lipid levels, which may confound the outcome of EVOO intake (Gaforio et al, 2019).

Results regarding recruiting female participants was widespread safety concerns for pregnant women and women who might become pregnant, resulting in women being left out of studies (Sabine 2011).Female and male differ, too, in terms of body weight and size, therefore dosing is also an critical consideration. Although it is less likely to affect the results in crossover trial study, women and men still have different levels or separate thresholds of certain biomarkers. In addition, there are also differences in the ways that men and women potentially experience many health conditions in terms of prevalence, symptoms, age at onset, and severity, including in the case of autoimmune diseases, such as lupus and multiple sclerosis and psychological disorders, such as depression and schizophrenia.

5.7.2.4 Limitations

This study is, however, not without limitations. Firstly, this study was an “open label” study that could not blind participants. However, this is a common situation in dietary interventions. Self-reporting may be another potential limitation since participants evidently underreported their food intake at baseline. However, good self-reported compliance together with changes in the outcomes of interest indicate that during the study this is likely to have been minimised. Thirdly, the dietary pattern between Caucasians and East Asians is likely to be culturally varying, especially for habitual dietary intake (Leung and Stanner 2011). Due to cultural differences between Western and Eastern diets, background usual diet has been recognized as a potential factor affecting the effectiveness of EVOO interventions, with most British following a westernized, rich in saturated fat diet while most East Asians following an Eastern diet with unhealthy oils into cooking, such as those containing trans-fat and animal fat (Chan, Malik et al. 2009). Evidence observed that the consumption of a high-cholesterol diet (HCD) with EVOO was responsible for increasing the TC and LDL-C when compared with the group receiving only HCD without the addition of another lipid source (Venturini, Simão et al. 2015). The inability to assess potential interactions between EVOO and other diet components that might affect the generalizability of the outcomes because of dietary differences among ethnicities as synergistic or antagonistic interactions existing between nutrients within dietary patterns (Tapsell, Neale et al. 2016).

It might exist slight bias regarding food intake reports from participants as EI:BMR ratio < 1.35 is shown underreporting for both ethnicities (1.23 for Caucasians; 1.15 for East Asians; $p=0.543$) (**Table 5.4**). Under-reporting of energy intake might affect estimates of nutrient intakes. Under-reporters are shown to consume fewer foods rich in fat, energy and all other nutrients than did the subjects whose EI:BMR within the normal range (Mirmiran et al, 2006).

However, no statistically significant difference of weight, BMI, handgrip strength and body composition, nutritional intake between Caucasians and East Asians ($p<0.05$) during washout, interventional period (**Table 5.4; Table 5.5**) suggesting of good compliance during the whole intervention showed that EI:BMR ratio does not affect the outcomes of the trial.

Although male only recruitment simplifies the design of the clinical trial, excluding female participants can be considered as a limitation since the awareness in order to reduce gender disparities in research and clinical care has been arising (Mosca, Barrett-Connor et al. 2011). The lack of inclusion of women in CVD trials has already been highlighted as a barrier to making clinical recommendations in the initial AHA evidence-based guidelines for the prevention of CVD in women (Mosca, Barrett-Connor et al. 2011). Evidence presented by (Mosca, Barrett-Connor et al. 2011) reported that the absolute numbers of female living with and dying of CVD and stroke exceed those of men, as does the number of hospital discharges for heart failure and stroke, although for both women and men, coronary heart disease is the largest contributor to CVD morbidity and mortality.

Study (Galiuto 2015) showed that while menopause, and some major cardiovascular risk factors such as systolic arterial hypertension, smoking, diabetes, TG and HDL-C levels mainly act in women. In males, cardiovascular risk profile linearly increases over time, along with atherosclerotic process continuously develops. On the contrary, females are protected from atherosclerosis in the fertile age, since estrogens exert beneficial effects on cardiovascular system, by acting via genomic and non-genomic mechanisms (Galiuto 2015). After menopause, estrogen deficiency leads to exponential increase of cardiovascular risk, as it induces structural and functional changes including endothelial dysfunction, imbalance of autonomic activity towards an increased adrenergic status, visceral adiposity, and enhanced systemic inflammation in cardiovascular system (Galiuto 2015). All these factors contribute to the development of systemic hypertension, abnormal lipid profile and insulin resistance. By this point of view, menopause itself can be represented as an independent predictor of CVD (Galiuto 2015) thus female participants should be recruited and targeted

for the future CVD clinical trials. Last limitation of the study is that the beneficial effect obtained in a group of healthy Caucasians and East Asians adults with low cardiovascular risk score, may be meaningless in other groups of risk.

5.7.3 Unanswered questions and future research

In this human trial study, bottles of EVOO was used for the dietary intervention, rather than olives products, or other grades (e.g., refined olive oil, virgin olive oil) or forms of olive oil (e.g. capsules), which are associated with different acceptability of daily intake and different bioavailability of MUFAs, PUFAs, and other minor phenolic compounds including phenols oleuropein and hydroxytyrosol (Marcelino, Hiane et al. 2019). The way of usage of liquid olive oil, such as cooking, dipping, marinading and baking, which are associated with different feasibility of daily olive oil consumption can be changed in future trial too (e.g. OO incorporated into buns) (Tholstrup, Hjerpsted et al. 2012). These different forms of olive oil could be evaluated and ascertain the effects of varying healthy fats does and their effects on both traditional and novel cardiovascular biomarkers and both resting BP and 24-hour BP. Further research could alter the length of intervention (e.g. 6 weeks to 9 weeks) (Oliveras-López, Molina et al. 2013, Galvão Cândido, Xavier Valente et al. 2018) and change the dose of EVOO intake (e.g. 50g; 60g) (Oliveras-López, Molina et al. 2013, Khaw, Sharp et al. 2018).

As already stated above, this study was conducted with male participants only, and so there would be room for future research to address whether the findings of this study could be replicated in women. Similarly, future research could investigate the impact of these intervention in middle-age groups from Caucasians and East Asians, at risk of, or affected by, cardiovascular disease or cardiovascular risk factors. The potential explanation for the difference of ambulatory BP and inflammatory biomarkers between East Asians and Caucasians after EVOO consumption would be worth to be explored. The further study will be expected to be determined by the different degree of digestion of tyrosol (T) and hydroxytyrosol (HT) from EVOO in urine samples of two ethnicities.

This study was designed to test a dose of EVOO that is commonly observed among individuals in Mediterranean countries, however it is uncertain whether such level of consumption (ie. 30 ml/day) could be sustainable in the long term, future research could

evaluate the effect of lower doses that are likely to be more acceptable and sustainable in the long term.

5.8 Conclusion

In conclusion, this study provided evidence on the benefits of the consumption of consistent habitual diet except standardising their lunch with extra virgin olive oil 30 ml compared with butter, for two weeks, has a significant impact on reducing TC, LDL-C, 24-hour SBP including daytime SBP, night-time DBP and night-time MAP in both Caucasians and East Asians participants with no risk of cardiovascular disease. However, total cholesterol, E-selectin, 24-hour SBP, daytime SBP and daytime MAP were improved among East Asians participants after EVOO intake while LDL-cholesterol, non-HDL cholesterol and night-time DBP was improved only among Caucasians. TC was improved after olive oil intake for all participants. Based on these findings, consumption of EVOO should be advocated to improve the cardiovascular health of Caucasians and East Asian individuals.

CHAPTER 6 General Discussion

6.1 Thesis Summary

The biological ageing process is influenced by lifestyle factors (75% contribution) and genetic factors (25% contribution) which accounts for a fact that dietary intake in lifestyle aspects, plays a critical role in determining healthy ageing (Passarino, De Rango et al. 2016). People become more dissimilar from their contemporaries of the same chronological age as they become older mostly due to dietary factors, so that personalized dietary interventions alongside recommendations is of vital important (Passarino, De Rango et al. 2016). Among the physiological and metabolic changes occurring with ageing, the ageing of heart function is the key determinant of health. The Mediterranean dietary pattern is well recognised as one of the main contributors related to improve cardiovascular health, which is one of the leading cause of mortality and mobility (Anand, Hawkes et al. 2015), even during recent years, a high prevalence of cardiovascular disease among people who experienced COVID-19 are reported (Bansal 2020, Clerkin, Fried et al. 2020) and pre-existing CVD is associated with worse outcomes among patients with COVID-19 (Aggarwal, Cheruiyot et al. 2020),

As for tackling cardiovascular health problems, DASH diet trials was not designed for CVD clinical events (Kwan, Wong et al. 2013) and there is no RCT which has evaluated the impact of the Portfolio Diet on CVD clinical events (Anand, Hawkes et al. 2015). Low-fat-high-carb (LFHC) diet unfortunately result in such a high glycaemic load on HDL-C and glucose metabolism (Scholl 2012). Additionally, there is insufficient research of meta-analysis on the Japanese diet regarding cardio-protective effects (Teramoto 2017) while the DASH diet was reported as having a poor compliance among a Western population (Kwan, Wong et al. 2013). Therefore, choosing the Mediterranean diet as the worldwide dietary recommendation amongst various dietary patterns is becoming the most popular and is being promoted in improving vascular health. The Mediterranean diet (Ruiz-Canela and Martínez-González 2011), which is characterised by high intake of vegetables, grain, nuts and especially olive oil, has received the most attention in relation to chronic disease prevalence, especially cardiovascular disease (Ruiz-Canela and Martínez-González 2011) as PREDIMED study suggested that a MD supplemented with either extra virgin olive oil or mixed nuts cut the risk of CVD events by as much as 30% in subjects at high risk of

developing heart diseases after 5-year follow-up (Estruch, Ros et al. 2013). Extra virgin olive oil, characterized as the main component of MD, is responsible for a large part of cardio benefits associated to the Mediterranean diet (Mazzocchi, Leone et al. 2019), in their randomized trial (Casas, Sacanella et al. 2014) reported that high adherence to a MD intervention supplemented with 50ml/d EVOO for 12-month period was associated with a significant decrease in inflammatory markers (C-reactive protein and interleukin-6), a reduction in SBP, DBP and plasma LDL-C concentration.

The main aim of this thesis was to investigate the effectiveness of extra virgin olive oil with habitual diet on cardiovascular biomarkers and cardiovascular risk factors in two ethnicities - East Asians and Caucasians living in the UK. This novel thesis topic was originating from the interest on evaluating the response to dietary interventions among different ethnic groups. This thesis followed a series of logical steps presented in separate chapters and is summarised in **Figure 6.1**.

In **Figure 6.1**, phase one of the present PhD programme involved an overview of the topic. In phase two, the acceptability, feasibility and frequency of olive oil consumption in Caucasians and East Asians based in Newcastle upon Tyne UK by Online Survey was researched. In phase three, the literature of systematic review and meta-analysis on the association between olive oil and nuts (two major components in the Mediterranean diet) and cardiovascular health in different ethnicities was researched.

In the survey chapter (**Chapter 2**), the response from East Asians overall reported a better health and the acceptability and the frequency of olive oil consumption in East Asians is better than Caucasians. Olive oil consumption in both ethnicities - 142 Caucasians (87.3% female; mean age: 32.8±SD12.2; BMI: 22.8±SD2.8) and 142 East Asians (78.2%; mean age: 32.8±SD10.9; BMI: 22.4±SD2.7), was closely positively associated with older age, higher MD score, higher MD acceptability and lower PBHE.

Healthy eating habits - the Mediterranean-style diet, emphasizing in extra virgin olive oil, nuts consumption, have given increasingly attention by global population as the benefits of olive oil and nuts intake on cardiovascular health have been evidenced numerously. The next step of this thesis was to evaluate the evidence from previous RCTs on the effects of olive oil and nuts on cardiovascular risk factors and cardiovascular biomarkers. Systematic review and meta-analysis in **Chapter 3** reported that nuts consumption significantly improve

biomarkers including TC, HDL-C, LDL-C, TG, VLDL-C, Apo B, FMD and FFA. The systematic review and meta-analysis on **Chapter 4** reported that olive oil significantly improve biomarkers such as PAI-1 and tPA.

Our systematic review and meta-analysis found that olive oil intake had beneficial effects on cardiovascular health. These results suggested the fact that such dietary habit could be one of the important modifiable factors affecting the maintenance of a healthy phenotype, which is low risk of any cardiovascular diseases and cardiovascular risk factors including hypertension, obesity, diabetes and high cholesterol. However, what cardiovascular biomarkers and risk factors would be changed/improved by daily olive oil intake in East Asians and Caucasians are worth of future clinical research.

Phase four of the present PhD programme was the development of dietary intervention on comparing EVOO and butter on cardiovascular risk factors in Caucasians and East Asians (**Figure 6.1**). In **Chapter 4** of systematic reviews and meta-analysis of the scientific literature reported that previous interventions on olive oil or olive oil capsules spending average duration about 5 weeks and olive oil doses were with daily consumption ranging between 13.3 and 64.8 grams. The results from systematic review, as a knowledge basis, help researchers to identify clinical outcome measures relevant to cardiovascular risk factors. In adherence to the gained knowledge, the selection criteria for the measures included biomarkers that responded to dietary interventions in my designed trial, particularly to EVOO. Thus, a tentative panel of traditional lipids profiles and inflammatory biomarkers were potentially selected to be tested in two ethnicities in our clinical trial.

Therefore, phase four of the present PhD programme was reported in **Chapter 5 (Figure 6.1)**, our crossover randomised controlled trial aimed to investigate the impact of extra virgin olive oil intervention on risk factors of cardiovascular health as well as markers of inflammatory biomarkers, blood lipids and other novel biomarkers have not been previously investigated. In addition, this RCT is novel since there are no previous RCTs investigating and comparing the effects of extra virgin olive oil and butter intake among East Asians and Caucasians living in Northeast England, UK.

The results of the extra virgin olive oil (EVOO) intervention on BP, blood lipids profiles and several inflammatory as well as novelty biomarkers were examined in **Chapter 5** and consumption of 30 ml of EVOO over a 2-week period produced a positive effect on 24-hour

SBP including daytime SBP, night-time DBP and MAP and TC, LDL-C for all participants. Furthermore, there is different effect between East Asians and Caucasians. East Asians participants showed a better effect on 24-hour SBP and daytime SBP, MAP while night-time DBP was improved among Caucasians after EVOO intake. EVOO intake also has a positive effect on blood lipids such as TC, circulating biomarkers such as E-selectin in East Asians while LDL-C and non-HDL are improved among Caucasians after EVOO intake.

Further investigation and research are still warranted since whether different types of olive oil containing different levels of nutritional benefits exert the same beneficial effect as EVOO remain unknown. For example, EVOO is not the only olive oil form available for consumption. The share of ordinary (not virgin) olive oil imported by the UK among the total British olive oil imports was 33%, followed by EVOO (28%) and VOO (19%) (CBI - Ministry of Foreign Affairs 2020). Two distinct types including refined and unrefined olive oil are widely distributed into the worldwide marketing could be studied in the near future. Moreover, it was believed that fish oil with dietary ω -3 PUFAs intake have beneficial effects on the decrease in TG levels (Belalcazar, David et al. 2010) and olive oil may act synergistically with fish oil by increasing the incorporation of ω -3 fatty acids in cell membranes (Crawford, Galli et al. 2000) as evidence showed that middle-aged individuals (mean age 51.45 ± 8.27 y) receiving 10 ml/day of EVOO plus 3 g/day of fish oil exert beneficial synergistic effects on lipid metabolism (Venturini, Simão et al. 2015).

6.2 Novelty and strength of the present thesis

The work presented in this thesis contributes to our understanding of the impact of food and diet on cardiovascular health. This work is novel in several ways and has a number of strengths. Strengths of this work include, adopting a rigorous methodology and a series of logical steps on investigating the effect of extra virgin olive oil on cardiovascular health. The design of the clinical trial presented here was developed based on evidence from previous relevant literature of systematic review and meta-analyses. Previous RCTs focused on investigating the effects of extra virgin olive oil (EVOO) intake on markers of cardiovascular risk but not been evaluated previously among two ethnicities – East Asians and Caucasians. The present work thus fills a gap in the literature by reporting results comparing the cardio health impacts between two ethnicities living near northeast of the UK. The result of the

clinical trial was consistent with previous evidence. In addition, this is the first clinical trial investigating the effects of liquid extra virgin olive oil (rich in MUFAs and phenolic compounds such as hydroxytyrosol, tyrosol, and oleuropein) on 24-hour BP plus novel biomarker such as syndcan-1, as well as other cardiovascular risk factors which have been less studied and published such as PAI-1, sICAM-1, sVCAM-1, sE-selectin and sP-selectin between Caucasians and East Asians. However, compliance, albeit good, was self-reported and there were no objective methods in place to measure compliance. Further research could determine tyrosol (T) and hydroxytyrosol (HT) in urine samples by gas chromatography-mass spectrometry, alongside self-report checklists to measure compliance (Miro-Casas et al, 2001).

6.3 Relevance for Stakeholders

The results of the present human trial are of relevance to healthcare professional such as nutritionists and dietitians who could possibly use these to encourage the adoption of a diet with an increased extra virgin olive oil intake to reduce BP and further reduce the risk of CVD. These may be useful specially for people with hypertension or borderline hypertension for East Asians and Caucasians, the intake of extra virgin olive oil (EVOO) should be encouraged and the positive effects of EVOO consumption should be highlighted.

6.4 Future work and public health implications for clinicians or policymakers

To the best of our knowledge, no previous RCTs have tested and compared the effect of EVOO on two different ethnicities living around Northeast England. The positive impacts of EVOO on resting blood pressure, 24-hour/ambulatory blood pressure, blood lipids and inflammatory biomarkers encourage further studies with a larger population to investigate the effects of EVOO consumption, as well as the influence of micro-components present in EVOO, such as fat component oleic acid (OA), polyphenols (oleuropein and hydroxytyrosol) and phenolic compounds, including the secoiridoids oleocanthal and oleacein in EVOO, which could act in a synergistic/antagonistic approach regulating the activity of these molecules.

The current evidence supports medium-term incorporation of 30ml of liquid EVOO daily into habitual diets is highly likely to reduce cardiovascular risk in the primary preventions of cardiovascular diseases in healthy individuals. Either healthy individuals with normal blood pressure or patients with high blood pressure who substitute 30ml of EVOO consumption known as antioxidant polyphenols in their diets replacing with other types of fat can help to keep blood pressure healthy or reduce the amount of medication.

The current clinical trial provides evidence to confirm and strengthen dietary recommendations to increase liquid EVOO in the diet as emphasised in Mediterranean diet recommendations (Martínez-González et al., 2019; Ditano-Vázquez, et al.,2019). The present work does not put restrictions on specific age range, the majority of healthy participants, however, are young-aged university students. Evidence suggested metabolism slows down around age 60 years as being less active, losing muscle mass, higher possibility of experiencing diseases, slower process of digestion and absorption of certain nutrients in EVOO, thus results in different effects after EVOO intake. Young aged (18-44 years old), middle-aged (45-65 years old) and older adults (aged 65 and above) will be targeted to compare and determine whether ambulatory blood pressure, blood lipids and biomarkers change with age, and whether or not moderate or high amounts of EVOO intake (30-35ml or more amounts) would improve blood lipids profiles as well as inflammatory biomarkers such as PAI-1, IL-6, sICAM-1, sVCAM-1 and CRP.

However, this clinical trial finding highlights the need for further elucidation of the more nuanced relationships between EVOO and cardiovascular health. In particular, a future human trial study on dietary interventions could target female individuals as well as individuals of both gender at high CVD risk. It would also be useful to evaluate other foods known to reduce CVD risks. The effects of other forms and types of olive oil with different doses (e.g., higher doses 40ml) on cardiovascular risk factors require further investigation. The findings cannot be extrapolated to all liquid olive oil products such as olive pomace oil, frylight olive oil spray. Further research is needed to determine the effects of EVOO on the cardiovascular health of different ethnicity in a larger number with high CVDs risk and female participants should also be included too.

The positive impacts of MUFAs, omega-3 PUFAs and phenolic compounds on 24-hour blood pressure, blood lipids levels and inflammatory biomarkers encourage further research with a larger population of both Caucasians and East Asians who live in the UK to

investigate the influence of EVOO alongside MUFA OA, phenolic compounds such as hydroxytyrosol, oleuropein, secoiridoids oleocanthal and oleacein, which could act in a synergistic/antagonistic way regulating the activity of these molecules.

6.5 Conclusion

As a conclusion, current human clinical trial supports the 30 ml of liquid EVOO improves cardiovascular health in East Asians and Caucasians, particularly in 24-hour BP and LDL-cholesterol, future clinical trial design will be highlighted in both genders, comparing different age groups and larger dose of EVOO cooperating with habitual diets in East Asians and Caucasians.

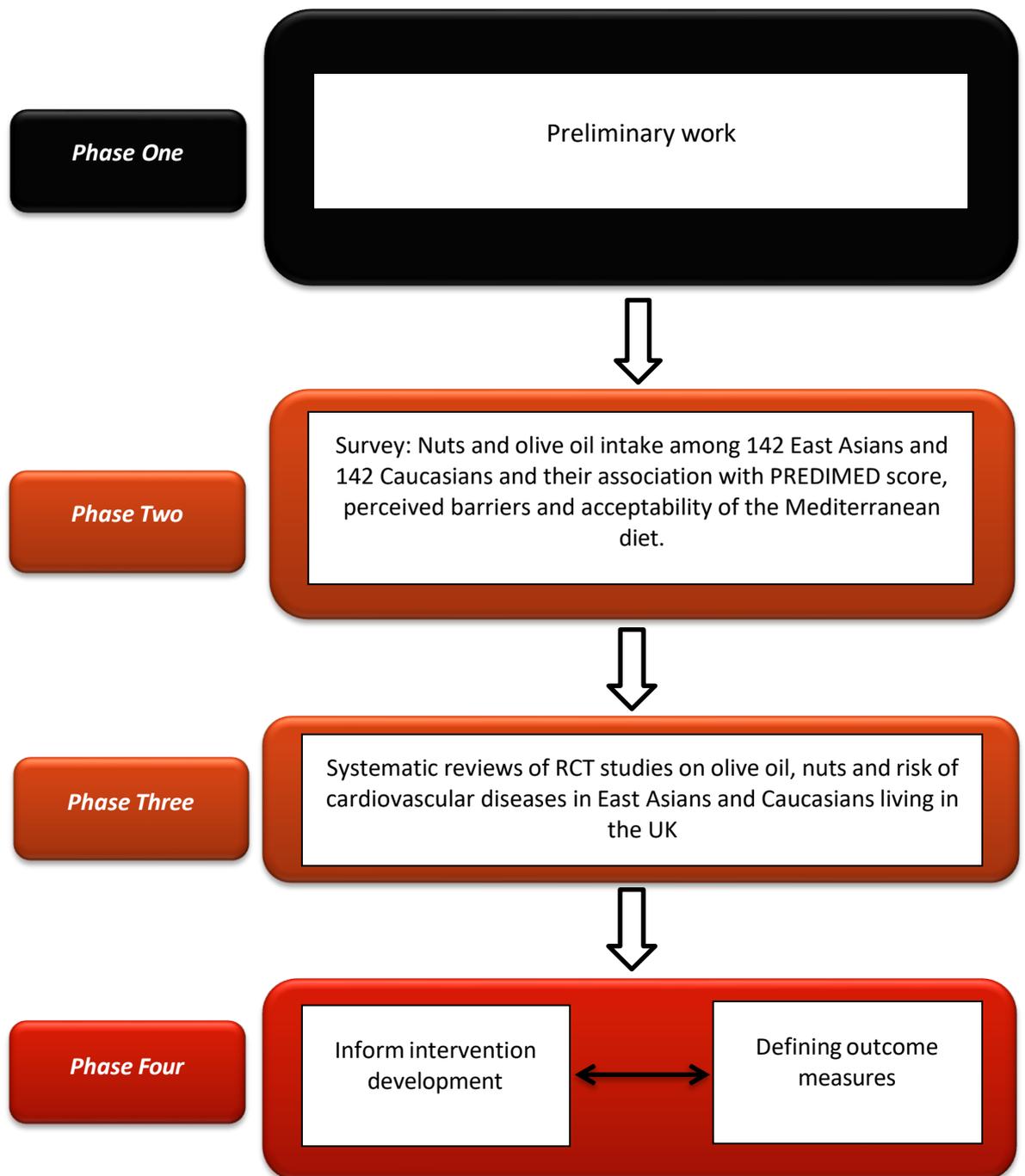


Figure 6.1 Overview of this PhD programme

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Appendix A

Supplementary information for **Chapter 2**

Appendix A1. Ethical Approval of the survey

Submission Ref: 892

Following independent peer review of the above proposal, I am pleased to inform you that **APPROVAL** has been granted on the basis of this proposal and subject to continued compliance with the University policies on ethics, informed consent, and any other policies applicable to your individual research. You should also have current Disclosure & Barring Service (DBS) clearance if your research involves working with children and/or vulnerable adults.

The University's Policies and Procedures are [here](#)

All researchers must also notify this office of the following:

- Any significant changes to the study design, by submitting an 'Ethics Amendment Form'
- Any incidents which have an adverse effect on participants, researchers or study outcomes, by submitting an 'Ethical incident Form'
- Any suspension or abandonment of the study.

Please check your approved proposal for any Approval Conditions upon which approval has been made.

Use this link to view the submission: [View Submission](#)

Research Ethics Home: [Research Ethics Home](#)

Please do not reply to this email. This is an unmonitored mailbox. Queries should be forwarded to ethicssupport@northumbria.ac.uk

Appendix A2. Email recruitment

Hello,

We're currently looking for male and female volunteers above 18 years of age who aren't full-time students.

Our PhD students, Louise Francis and Fan Liang in the department of Applied Sciences at Northumbria University is currently recruiting participants for an online survey about weight loss attempts and adherence to the Mediterranean diet among adults in Northeast England.

This study has been reviewed and received full ethical approval from the Faculty of Health and Life Sciences/Northumbria University Research Ethics Committee.

The survey involves completing a questionnaire on basic background information about eating habits and **it should take no longer than 15 minutes** to complete.

If you would like to take part, please visit the following link:

<https://northumbria.onlinesurveys.ac.uk/public-opinions-of-the-mediterranean-diet-copy>



**Public opinions of the
Mediterranean diet
northumbria.onlinesurveys.ac.uk
Online survey BOS**

However, **if you wish to discuss any aspect of this study before taking part in it please contact** Louise Francis and Fan Liang at louise.francis@northumbria.ac.uk; fan.liang@northumbria.ac.uk

Please feel free to forward this email to anyone that you think might be interested in taking part in this study.

Best wishes,

Louise Francis, Fan Liang and Jose Lara

Appendix A3. Survey

Public opinions of the Mediterranean diet (Asian) (copy)

INFORMATION SHEET

Researchers: Louise Francis, Fan Liang

Contact details: louise.francis@northumbria.ac.uk, fan.liang@northumbria.ac.uk

Supervisor: Dr Jose Lara

Contact details: jose.lara@northumbria.ac.uk

What is the purpose of this project?

This research forms part of a PhD project looking at the feasibility and acceptability of the Mediterranean diet in the UK. It is therefore important to assess the views of the public on this dietary pattern. This information will inform future clinical trials aiming to assess the impact of a combination of Mediterranean diet consumption and intermittent fasting on weight loss and cardiovascular biomarkers.

Why have I been selected to take part?

You have been invited to complete an online survey as you meet the following inclusion criteria: above 18 years of age, a UK resident and an English-speaker as the survey is written in English and no translational facilities are available.

What will I have to do?

You will be asked to complete an anonymous online survey which should take around 15 minutes. The survey will involve questions about background information such as age and height, questions about your opinions of a novel diet, and questions about how often you eat certain foods. You can save the survey part way through and complete it at another time.

Will my participation involve any physical or psychological discomfort?

Participating in this research should not involve any physical or psychological discomfort.

How will confidentiality be assured?

In order to maintain anonymity, an online survey is used which does not collect any personal data such as your name or home address. All data will be treated in accordance with the Data Protection Act, and data will only be used by members of the research team for reasons appropriate to the research question.

Has this research received appropriate ethical clearance?

This study has been given full ethical approval by the Northumbria University Ethics Committee.

Will I receive any financial reward for taking part?

No financial rewards are offered for participating in this study.

How can I withdraw from the project?

Participants are not able to withdraw from the project after their survey has been submitted as the software used does not allow identification of specific participants' data. However if you choose to withdraw from the study before the survey has been submitted, closing the browser window in which the survey is open will terminate the survey and no data will be collected.

I would like further information or to ask a question. Who do I contact and how?

For any further information, do not hesitate to contact the investigator (Louise Francis and Fan Liang) or the project supervisor (Dr Jose Lara) using the email addresses at the top of this information sheet.

If you have any questions, please click 'Finish later' and return to this survey once your questions have been answered.

CONSENT FORM

I consent to participate in this survey, and have read and understood the Information Sheet. (Required)

Yes

No

BACKGROUND QUESTIONS

What is your gender? (Required)

Male
 Female
 Prefer not to say

What was your age on your last birthday? (Required)

What is your ethnicity? (Required)

Caucasian
 East Asian (Chinese origin)
 South Asian (non-Chinese origin)
 Black
 Other

If you selected Other, please specify:

Are you originally from a Mediterranean country (e.g. Spain, Italy, Greece etc)?

Yes

No

Other

If you selected Other, please specify:

Where in the UK do you currently reside? (Required)

- North East England
- North West England
- South East England
- South West England
- Scotland
- Wales
- Northern Ireland
- Other

If you selected Other, please specify:

What is your marital status? (Required)

Single

Married

Widowed/Divorced/Separated

Prefer Not to say

Other

If you selected Other, please specify:

What is your employment status? (Required)

- Unemployed
- Working part-time (less than 30 hours per week)
- Working full-time (more than 30 hours per week)
- Carer/Housewife/Househusband
- Retired
- Prefer not to say
- Full-time student
- Part-time student
- Other

If you selected Other, please specify:

What is your highest education level? (Required)

None GCSE/O-Level

A-Level Diploma

Foundation degree

Undergraduate degree

- Postgraduate
- degree
- PhD/equivalent
- Prefer not to say
- Other

If you selected Other, please specify:

Are you currently a smoker, an ex-smoker, or have you never smoked? *Required*

- Current smoker
- Ex-smoker
- Never smoked

Do you currently drink any alcohol? *Required*

- Yes
- No

What is your weight in kilograms? Click [here](#) to open a unit conversion website in a new window – you may find it useful if you need to convert your weight in stones and pounds to kilograms. *Required*

What is your height in metres?

Click here to open a unit conversion website in a new window -

you may find it useful if you need to convert your height from feet and inches to metres. *(Required)*

Do you have a current diagnosis for any of the following?

	<i>Required</i>	
	Yes	No
Cardiovascular disease	<input type="radio"/>	<input type="radio"/>
Hypertension	<input type="radio"/>	<input type="radio"/>
Type 2 diabetes	<input type="radio"/>	<input type="radio"/>
High cholesterol	<input type="radio"/>	<input type="radio"/>

Do you follow a special diet because of a medical condition? *(Required)*

Yes

No

What health condition is your diet tailored to?

WEIGHING YOURSELF

Do you have a set of scales for weighing yourself at home? *Required*

- Yes
- No

How often do you weigh yourself/have somebody else weigh you? *Required*

- Never
- Yearly
- Monthly
- Weekly
- Daily Other

If you selected Other, please specify:

At the present time, are you trying to lose weight, trying to gain weight, or not trying to change your weight? *Required*

- Trying to lose weight
- Trying to gain weight
- Not trying to change weight

Have you ever attempted to lose weight in the past? Required

Yes

No

YOUR WEIGHT LOSS EXPERIENCE

How many separate weight loss attempts have you made in the past? Required

How many of these would you say were successful? Required

Have you used any of the following in your attempt(s) to lose weight?

	Yes	No
Diet plans involving group meetings (e.g. Slimming World, WeightWatchers)	<input type="checkbox"/>	<input type="radio"/>
Other diet plans (e.g. Lean in 15)	<input type="radio"/>	<input type="radio"/>
Exercise plans (e.g. fitness DVDs)	<input type="radio"/>	<input type="radio"/>
Meal replacements (e.g. Slim Fast)	<input type="radio"/>	<input type="radio"/>
Medicinal aids (e.g. slimming tablets)	<input type="radio"/>	<input type="radio"/>
Fasting (e.g. 5:2 diet)	<input type="radio"/>	<input type="radio"/>
Surgical methods (e.g. gastric band)	<input type="radio"/>	<input type="radio"/>

YOUR OWN DIET

How many times do you usually eat out **per month**? (i.e. consume food not cooked at home).
Please give a number. *Required*

When eating out, which of the following cuisines do you prefer? (Please choose one) *Required*

- Indian
- Chinese
- Italian
- Spanish
- Mexican
- British
- American
- Other

If you selected Other, please specify:

Do you ever use olive oil in your diet? *Required*

- Yes
- No

How much olive oil do you use per day, in approximate tablespoons?

THE MEDITERRANEAN DIET

Which of the following statements is most true for you? Required

- I've never heard of the Mediterranean diet
- I know a little bit about the Mediterranean diet
- I know quite a bit about the Mediterranean diet
- I fully understand the concept of the Mediterranean diet
- Other

If you selected Other, please specify:

Do you believe that your usual diet is healthy? Required

- Yes
- No
- Prefer not to say
- Other

If you selected Other, please specify:

Are any of the following factors barriers to your healthy eating?

Please don't select more than 1 answer(s) per row.

Please select at least 20 answer(s).

	Yes	No

Irregular working hours	<input type="checkbox"/>	<input type="checkbox"/>
Busy lifestyle	<input type="checkbox"/>	<input type="checkbox"/>
Giving up foods that I like	<input type="checkbox"/>	<input type="checkbox"/>
Lack of willpower	<input type="checkbox"/>	<input type="checkbox"/>
I don't want to change my eating habits	<input type="checkbox"/>	<input type="checkbox"/>
Limited cooking skills	<input type="checkbox"/>	<input type="checkbox"/>
Healthy food is more perishable	<input type="checkbox"/>	<input type="checkbox"/>
Lengthy preparation of healthy food	<input type="checkbox"/>	<input type="checkbox"/>
Limited storage facilities	<input type="checkbox"/>	<input type="checkbox"/>
Limited cooking facilities	<input type="checkbox"/>	<input type="checkbox"/>
Increased price of healthy foods	<input type="checkbox"/>	<input type="checkbox"/>
Unappealing healthy food	<input type="checkbox"/>	<input type="checkbox"/>
Strange or unusual healthy foods	<input type="checkbox"/>	<input type="checkbox"/>
Feeling conspicuous among others	<input type="checkbox"/>	<input type="checkbox"/>
Taste preferences of family and friends	<input type="checkbox"/>	<input type="checkbox"/>
Not knowing enough about healthy eating	<input type="checkbox"/>	<input type="checkbox"/>
Experts keep changing their minds about healthy foods	<input type="checkbox"/>	<input type="checkbox"/>
Limited healthy choice when I eat out	<input type="checkbox"/>	<input type="checkbox"/>
Healthy options not available in shop or canteen at work	<input type="checkbox"/>	<input type="checkbox"/>
Not enough healthy food to satisfy hunger	<input type="checkbox"/>	<input type="checkbox"/>

THE MEDITERRANEAN DIET

Would you find the following Mediterranean dietary guidelines acceptable?

(If you don't eat or drink something, e.g. meat or wine, please just click 'no')

	Yes	No	I don'tknow
Olive oil should be used as the main culinary fat source, replacingbutter etc	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
At least 4 tablespoons of olive oil should be consumed per day	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Red meat should only be consumed a maximum of twice per week(1 serving = 70g, or three slices of ham)	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Meats such as chicken, turkey and rabbit should be chosen overbeef, pork or veal	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Sweets such as desserts, chocolate and anything else high in refined sugars should be consumed a maximum of 3 times per week	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Carbonated or sweet drinks should be consumed a maximum ofonce per day (1 serving = 150ml)	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
A minimum of 2 servings of vegetables (excluding white potatoes)should be eaten per day (1 serving = 80g, or 2 broccoli florets, three heaped tablespoons of cooked vegetables such as carrots, or 3 celery sticks)	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
A minimum of 3 servings of fruit should be eaten per day (1 serving = 80g, or 1 banana, 2 kiwis, or 1 heaped tablespoon of sultanas)	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Butter, margarine and cream (1 serving = 10g) should be eatenless than once per day	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
7 glasses of wine should be consumed per week (1 glass = 125ml)	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
More than 3 servings of legumes (e.g. kidney beans, lentils orchickpeas) should be consumed per week (1 serving = 5 tablespoons of cooked food)	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
More than 3 servings of fish or shellfish should be consumed per week (1 serving of fish = 140g, or about the size of a cheque book)	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>

More than 3 servings of unsalted nuts should be consumed perweek (1 serving = 40g, or a small handful)	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
A meal containing vegetables, pasta or rice served with a sauce made using tomato, garlic, olive oil and onion should be eaten atleast twice per week	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>

APPENDIX A4.

DEBRIEF SHEET

Researcher: Louise Francis, Fan Liang

Contact details: louise.francis@northumbria.ac.uk, fan.liang@northumbria.ac.uk

Supervisor: Dr Jose Lara

Contact details: jose.lara@northumbria.ac.uk

What was the aim of this project?

This project aimed to investigate the opinions of the public on the acceptability and feasibility of the Mediterranean diet in the UK.

Have I been deceived in any way during the study?

No deception was used in this study.

How will I find out about the results?

Should you wish to receive information on the results of this study, please email one of the research team using the above email addresses.

What will happen to the information I have provided?

All data will be stored securely and confidentially until a certain time after the study has finished, when it will be destroyed.

How will the results be disseminated?

This research is intended for a PhD project and therefore results may be disseminated in a scientific journal.

How would I withdraw my information after finishing the study?

Participants are not able to withdraw from the project after their survey has been submitted as the software used does not allow identification of specific participants' data. However if you choose to withdraw from the study before the survey has been submitted, closing the browser window in which the survey is open will terminate the survey and no data will be

collected.

How can I register a complaint about this research?

If you have any worries about the way this study was carried out please do not hesitate to contact one of the research team using the provided email addresses.

THANK YOU

Thank you for your participation.

If you would like to be contacted about future related studies, please email louise.francis@northumbria.ac.uk or jose.lara@northumbria.ac.uk or fan.liang@northumbria.ac.uk

Appendix A5

STROBE Statement— STROBE 2007 (v4) checklist of items to be included in reports of observational studies in Survey* Checklist for cohort, case-control, and cross-sectional studies (combined).

Section/Topic	Item #	Recommendation	Reported on page #
Title and abstract	1	(a) Indicate the study's design with a commonly used term in the title or the abstract	54
		(b) Provide in the abstract an informative and balanced summary of what was done and what was found	54
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	55-59
Objectives	3	State specific objectives, including any prespecified hypotheses	60-61
Methods			
Study design	4	Present key elements of study design early in the paper	61
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	61
Participants	6	(a) <i>Cohort study</i> —Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up <i>Case-control study</i> —Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls <i>Cross-sectional study</i> —Give the eligibility criteria, and the sources and methods of selection of participants	61
		(b) <i>Cohort study</i> —For matched studies, give matching criteria and number of exposed and unexposed <i>Case-control study</i> —For matched studies, give matching criteria and the number of controls per case	N/A
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	61-63
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	62

Bias	9	Describe any efforts to address potential sources of bias	N/A
Study size	10	Explain how the study size was arrived at	61
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	62-63
Statistical methods	12	(a) Describe all statistical methods, including those used to control for confounding	N/A
		(b) Describe any methods used to examine subgroups and interactions	N/A
		(c) Explain how missing data were addressed	N/A
		(d) <i>Cohort study</i> —If applicable, explain how loss to follow-up was addressed <i>Case-control study</i> —If applicable, explain how matching of cases and controls was addressed <i>Cross-sectional study</i> —If applicable, describe analytical methods taking account of sampling strategy	N/A
		(e) Describe any sensitivity analyses	63

Results	Item #	Recommendation	Reported on page #
Participants	13*	(a) Report numbers of individuals at each stage of study — e.g., numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	63
		(b) Give reasons for non-participation at each stage	N/A
		(c) Consider use of a flow diagram	N/A
Descriptive data	14*	(a) Give characteristics of study participants (e.g., demographic, clinical, social) and information on exposures and potential confounders	63
		(b) Indicate number of participants with missing data for each variable of interest	N/A
		(c) <i>Cohort study</i> —Summarise follow-up time (e.g., average and total amount)	N/A
Outcome data	15*	<i>Cohort study</i> —Report numbers of outcome events or summary measures over time	63-72
		<i>Case-control study</i> —Report numbers in each exposure category, or summary measures of exposure	N/A

		<i>Cross-sectional study</i> —Report numbers of outcome events or summary measures	N/A
Main results	16	(a) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (e.g., 95% confidence interval). Make clear which confounders were adjusted for and why they were included	64-72
		(b) Report category boundaries when continuous variables were categorized	N/A
		(c) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	N/A
Other analyses	17	Report other analyses done— e.g., analyses of subgroups and interactions, and sensitivity analyses	N/A
Discussion			
Key results	18	Summarise key results with reference to study objectives	72-76
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	N/A
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	72-77
Generalisability	21	Discuss the generalisability (external validity) of the study results	72-77
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	N/A

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at <http://www.plosmedicine.org/>, Annals of Internal Medicine at <http://www.annals.org/>, and Epidemiology at <http://www.epidem.com/>). Information on the STROBE Initiative is available at www.strobe-statement.org.

Appendix A6

Checklist for Reporting Results of Internet E-Surveys (CHERRIES)		
Item Category	Checklist Item	Explanation
Design		
	Describe survey design	142 Caucasian and 142 Asian responses from UK and Shanghai, China were recruited and analysed in this online survey study. Participants have regular access to internet and email and, no other inclusion/exclusion criteria were employed. Recruitment was supported by Northumbria University. This online survey was advertised among staffs and students at Northumbria University. A “snowball” sampling procedure was attempted by requesting participants to forward the invitation to join the study to others. Participants of this study were recruited in a wide range of individuals in terms of education, residence, marital and socioeconomic status, age and ethnicity.
IRB (Institutional Review Board) approval and informed consent process		
	IRB approval	This study was approved by the Northumbria University ethics committee (Registration no. 000892)
	Informed consent	Researchers Louise Francis, Fan Liang and Jose Lara created this survey for academic research purpose of the feasibility and acceptability of the Mediterranean diet in the UK. After people click on the link of this online survey, the first page is the consent form alongside with the information sheet, and this online survey takes no longer than 15 minutes to complete.
	Data protection	In order to maintain anonymity, an online survey is used which does not collect any personal data such as your name or home address. All data will be treated in accordance with the Data Protection Act, and data will only be used by members of the research team for reasons appropriate to the research question.
Development and pre-testing		
	Development and testing	All questions in this online survey were discussed and created by researchers Louise, Fan and our supervisor Jose Lara. Few tempts of pilot survey responses send to lecturers and friends prior to publishing to the public in order to gain feedbacks.

Recruitment process and description of the sample having access to the questionnaire		
	Open survey versus closed survey	Our online survey is an open survey, which only need participants to be above 18 years of age, a UK resident and an English-speaker as the survey is written in English and no translational facilities are available.
	Contact mode	The initial contact with the potential participants was made on the Internet and also by Northumbria university email.
	Advertising the survey	Recruitment was supported by Northumbria University. The study was also advertised among staffs and students at Northumbria University. A “snowball” sampling procedure was attempted by requesting participants to forward the invitation to join the study to others.
Survey administration		
	Web/E-mail	All Online data from all responses in this online survey were put manually into an Excel database by researchers. After cleaning all the database in excel, all data were input into SPSS to analyse.
	Context	Online surveys (formerly BOS) is regarded as a powerful, flexible online survey which is an tool designed for Academic Research, Education and Public Sector organisations.
	Mandatory/voluntary	This online survey is a voluntary survey to be filled by every visitor who clicked “yes” in the consent form page on the website.
	Incentives	None
	Time/Date	The data was collected from August 2017 to April 2018
	Randomization of items or questionnaires	No randomisation of items was used.
	Adaptive questioning	Certain items were populated based on previous responses. The questionnaire online survey was shown in Appendix A3.

	Number of Items	This online questionnaire survey has 1-3 items per page.
	Number of screens (pages)	This online questionnaire survey was distributed by 16 pages.
	Completeness check	All survey items were deemed to be mandatory, and respondents prompted to complete outstanding items before leaving the survey page on which the item was contained. This online survey technically had been done the consistency or completeness checks before the questionnaire is submitted. All items provide a non-response option such as “not applicable” or “rather not say”, and selection of one response option should be enforced (Appendix A3).
	Review step	Respondents were able to review and change their answers through a back button which displays a summary of the responses and asks the respondents if they are correct.
Response rates		
	Unique site visitor	Determination of unique visitors was handled by the research team. Digital fingerprinting for geo-IP ensure that respondents only compete the survey once.
	View rate (Ratio of unique survey visitors/unique site visitors)	None
	Participation rate (Ratio of unique visitors who agreed to participate/unique first survey page visitors)	All participants (n=284) visited the survey informed consent page and started to do the online survey questionnaire. The recruitment rate is 100%.

	Completion rate (Ratio of users who finished the survey/users who agreed to participate)	All participants (n=284) completed this questionnaire online survey. The completion rate is 100%.
Preventing multiple entries from the same individual		
	Cookies used	N/A
	IP check	Digital fingerprinting for geo-IP ensure that respondents only compete the survey once.
	Log file analysis	N/A
	Registration	N/A
Analysis		
	Handling of incomplete questionnaires	All questionnaires had been completed and analysed.
	Questionnaires submitted with an atypical timestamp	N/A
	Statistical correction	All data collected from the Online survey were input into the SPSS to analyse.

Appendix A7.

2.3.5. The association between foods consumption and socio-demographic characteristics in East Asians and Caucasians

There is no statistically significant difference of marital status between both Caucasians and East Asians consume either walnuts or olive oil as p value are more than 0.05 (**Table 2.6; Table 2.6.1**). As shown in **Table 2.6.2**, the majority of Caucasians who resided in North East England are not walnuts consumers ($p=0.005$). Northeast English are not walnuts consumers compared to other regions ($p=0.005$). In **Table 2.6.4**, there is no significant difference between people who consume walnuts and people who do not consume walnuts concerning their education level as p-value are both higher than 0.05. There is no significant difference between either Caucasians or East Asians who consume olive oil and people who do not consume olive oil concerning their education level as p-value are both higher than 0.05.

Table 2.6 The association between walnuts intake and marital status in East Asians and Caucasians

Ethnicity			Walnuts		Total	Asymptotic Significance (2-sided)
			No	Yes		
Caucasia n	Marital	Single	77	8	85	.594
		Married	29	7	36	
		Divorced/widowe d/separated	10	2	12	
		Prefer not to say	2	0	2	
		Other	6	1	7	
	Total		124	18	142	
East Asian	Marital	Single	30	22	52	.885
		Married	35	21	56	
		Divorced/widowe d/separated	3	3	6	
		Prefer not to say	6	3	9	
		Other	13	6	19	
	Total		87	55	142	

Chi-Square Tests

Table 2.6.1 The association between olive oil consumption and marital status in East Asians and Caucasians

Ethnicity			Olive oil		Total	Asymptotic Significance (2-sided)
			No	Yes		
Caucasian	Marital	Single	26	59	85	.061
		Married	4	32	36	
		Divorced/widowed /separated	3	9	12	
		Prefer not to say	1	1	2	
		Other	4	3	7	
	Total		38	104	142	
East Asian	Marital	Single	35	17	52	.981
		Married	39	17	56	
		Divorced/widowed /separated	4	2	6	
		Prefer not to say	7	2	9	
		Other	13	6	19	
	Total		98	44	142	

Chi-Square Tests

Table 2.6.2 The association between walnuts intake and geography in East Asians and Caucasians

Ethnicity			Walnuts		Total	Asymptotic Significance (2-sided)
			No	Yes		
Caucasian	Residence	North East England	112	12	124	0.005
		Other	12	6	18	
	Total		124	18	142	
East Asian	Residence	North East England	25	12	37	0.360
		Other	62	43	105	
	Total		87	55	142	
Ethnicity			Olive oil		Total	Asymptotic
			No	Yes		

						Significance (2-sided)
Caucasian	Residence	North East England	31	93	124	.214
		Other	7	11	18	
	Total	38	104	142		
East Asian	Residence	North East England	21	16	37	.061
		Other	77	28	105	
	Total	98	44	142		

Chi-Square Tests

Table 2.6.3 The association between walnuts and olive oil intake and employment status in East Asians and Caucasians

Ethnicity			Walnuts		Total	Asymptotic Significance (2-sided)
			No	Yes		
Caucasian	Employment	Unemployed	2	1	3	.081
		Working part-time	28	10	38	
		Working full-time	16	2	18	
		Carer/housewife/househusband	1	0	1	
		Full-time student	69	5	74	
		Part-time student	5	0	5	
		Other	3	0	3	
		Total	124	18	142	
East Asian	Employment	Working part-time	4	1	5	.382
		Working full-time	51	29	80	
		Carer/housewife/househusband	5	4	9	
		Retired	3	6	9	
		Full-time student	20	10	30	
		Part-time student	1	0	1	
		Prefer not to say	2	2	4	
		Other	1	3	4	
Total	87	55	142			

Ethnicity			Olive oil		Total	Asymptotic Significance (2-sided)
			No	Yes		
Caucasian	Employment	Unemployed	0	3	3	.331
		Working part-time	8	30	38	
		Working full-time	5	13	18	
		Carer/housewife/househusband	0	1	1	
		Full-time student	25	49	74	
		Part-time student	0	5	5	
		Other	0	3	3	
	Total		38	104	142	
East Asian	Employment	Working part-time	3	2	5	.561
		Working full-time	58	22	80	
		Carer/housewife/househusband	7	2	9	
		Retired	6	3	9	
		Full-time student	20	10	30	
		Part-time student	1	0	1	
		Prefer not to say	1	3	4	
	Other	2	2	4		
Total		98	44	142		

Chi-Square Tests

Table 2.6.4 The association between walnuts and olive oil consumption and educational level in East Asians and Caucasians

Ethnicity			Walnuts		Total	Asymptotic Significance (2-sided)
			No	Yes		
Caucasian	Coded Education	None	1	0	1	0.939
		Secondary school	20	2	22	
		University	101	16	117	
		Prefer not to say	1	0	1	
		Other	1	0	1	
Total			124	18	142	
East Asian	Coded Education	University	84	55	139	0.380
		Prefer not to say	2	0	2	
		Other	1	0	1	
		Total	87	55	142	
Ethnicity			Olive oil		Total	Asymptotic Significance (2-sided)
			No	Yes		
Caucasian	Coded Education	None	1	0	1	.174
		Secondary school	7	15	22	
		University	29	88	117	
		Prefer not to say	0	1	1	
		Other	1	0	1	
Total			38	104	142	.210
East Asian	Coded Education	University	96	43	139	
		Prefer not to say	2	0	2	
		Other	0	1	1	
		Total	98	44	142	

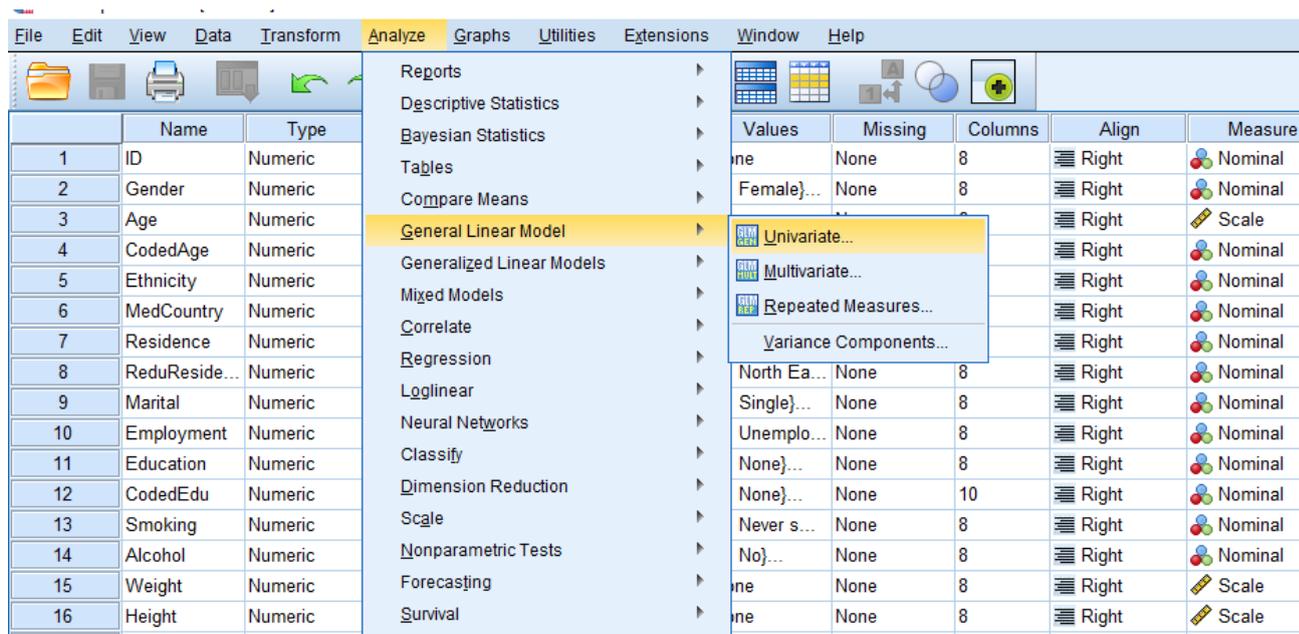
Chi-Square Tests

Appendix A8.

662 responses with East Asians and Caucasians completed an anonymous Bristol online Survey (BOS) named “Public Opinions of the Mediterranean Diet” with two-step cluster analysis, t-tests and Chi-square tests analyzing data. Adherence to MD as measured by a 14-item PREDIMED score is higher for East Asians than Caucasians. It shows that East Asians’ diet habits is healthier than Caucasians’ although Caucasians consume olive oil more frequently than Asians do but less amount than the Asian who eat olive oil.

Caucasians (n=151) were youngest ($25.6 \pm SD 8.8$), overweight ($26.6 \pm SD 7.9$), and reported the lowest MDPS ($4.3 \pm SD 1.7$), the largest score of PBHE ($10.6 \pm SD 2.3$), the lowest MD acceptability ($8.8 \pm SD 2.7$) and most frequency to dine out ($9.3 \pm SD 12.9$). Members of cluster-1 constituting 74.7% East Asians and 56.5% Caucasians (n=421) were the oldest ($30.7 \pm SD 12$) and the leanest group ($23.9 \pm SD 12.1$). Cluster-1 group was associated with a highest consumption of olive oil (60.8%), lowest number of PBHE ($0.01 \pm SD 0.08$), scored highest in MDPS ($6.5 \pm SD 1.9$) and reported least frequency of eating out ($6.4 \pm SD 6.9$).

However, in **Chapter 2**, univariate Analysis of Variance, which is used to analyze associations between two ethnicities (East Asians: n=142; Caucasians: n=142) with similar age and BMI from previous survey with total 662 responses.



Clusters

Input (Predictor) Importance
 ■ 1.0 ■ 0.8 ■ 0.6 ■ 0.4 ■ 0.2 ■ 0.0

Cluster	1	3	2
Label			
Description			
Size	60.1% (421)	21.5% (151)	18.4% (129)
Inputs	Willpower No (100.0%)	Price Yes (75.5%)	Busylifestyle Yes (76.0%)
	Busylifestyle No (100.0%)	Perishable Yes (61.6%)	Workinghours Yes (66.9%)
	Givingup No (99.5%)	Diningout Yes (67.5%)	Willpower Yes (72.9%)
	Preptime No (100.0%)	Willpower Yes (86.8%)	Givingup Yes (60.5%)
	Workinghours No (100.0%)	Preptime Yes (73.5%)	Preptime No (56.6%)
	Price No (100.0%)	Unappealing Yes (60.9%)	Habits No (82.2%)
	Diningout No (100.0%)	Satisfaction Yes (64.9%)	Price No (65.1%)
	Satisfaction No (100.0%)	Taste Yes (56.3%)	Satisfaction No (69.8%)
	Unappealing No (100.0%)	Knowledge No (55.6%)	Cookingskills No (79.8%)
	Perishable No (100.0%)	Workfood Yes (51.7%)	Diningout No (72.1%)
	Taste No (99.8%)	Givingup Yes (71.5%)	Conspicuous No (91.5%)
	Workfood No (100.0%)	Storagefacil No (58.3%)	Workfood No (79.1%)
	Cookingskills No (100.0%)	Busylifestyle Yes (74.2%)	Unappealing No (76.7%)
	Knowledge No (100.0%)	Experts No (63.6%)	Strangefoods No (88.4%)
	Storagefacil No (100.0%)	Cookingskills No (58.3%)	Taste No (81.4%)
	Experts No (100.0%)	Cookingfacil No (70.2%)	Knowledge No (90.7%)
	Strangefoods No (100.0%)	Strangefoods No (67.5%)	Perishable No (82.2%)
	Habits No (100.0%)	Workinghours Yes (52.3%)	Cookingfacil No (93.0%)
	Cookingfacil No (100.0%)	Habits No (78.1%)	Experts No (89.9%)
	Conspicuous No (100.0%)	Conspicuous No (86.8%)	Storagefacil No (89.1%)

Appendix B

Supplementary information for Chapter 3

Table B1: PRISMA Checklist

Section/topic	#	Checklist item	Reported on page #
TITLE			
Title	1	Identify the report as a systematic review, meta-analysis, or both.	78
ABSTRACT			
Structured summary	2	Provide a structured summary including, as applicable: background; objectives; data sources; study eligibility criteria, participants, and interventions; study appraisal and synthesis methods; results; limitations; conclusions and implications of key findings; systematic review registration number.	78 - 79
INTRODUCTION			
Rationale	3	Describe the rationale for the review in the context of what is already known.	80
Objectives	4	Provide an explicit statement of questions being addressed with reference to participants, interventions, comparisons, outcomes, and study design (PICOS).	81 - 82
METHODS			
Protocol registration and	5	Indicate if a review protocol exists, if and where it can be accessed (e.g., Web address), and, if available, provide registration information including registration number.	82 - 83
Eligibility criteria	6	Specify study characteristics (e.g., PICOS, length of follow-up) and report characteristics (e.g., years considered, language, publication status) used as criteria for eligibility, giving rationale.	83 - 84
Information sources	7	Describe all information sources (e.g., databases with dates of coverage, contact with study authors to identify additional studies) in the search and date last searched.	83 - 84
Search	8	Present full electronic search strategy for at least one database, including any limits used, such that it could be repeated.	82 - 83
Study selection	9	State the process for selecting studies (i.e., screening, eligibility, included in systematic review, and, if applicable, included in the meta-analysis).	82 - 83

Data collection process	10	Describe method of data extraction from reports (e.g., piloted forms, independently, in duplicate) and any processes for obtaining and confirming data from investigators.	84 - 85
Data items	11	List and define all variables for which data were sought (e.g., PICOS, funding sources) and any assumptions and simplifications made.	86 - 87
Risk of bias in individual studies	12	Describe methods used for assessing risk of bias of individual studies (including specification of whether this was done at the study or outcome level), and how this information is to be used in any data synthesis.	87 - 97
Summary measures	13	State the principal summary measures (e.g., risk ratio, difference in means).	87 - 97
Synthesis of results	14	Describe the methods of handling data and combining results of studies, if done, including measures of consistency (e.g., I^2) for each meta-analysis.	87 - 97

Figure B2. Meta-analysis of studies consuming nuts on FMD (%).

Ten studies, including 377 participants, evaluated the impact of nut consumption on FMD. Overall, nut consumption significantly increased FMD by 0.74 (95% CI 0.09 to 1.39; $p = 0.03$). Heterogeneity levels assessed by the I^2 test were low at 5%.

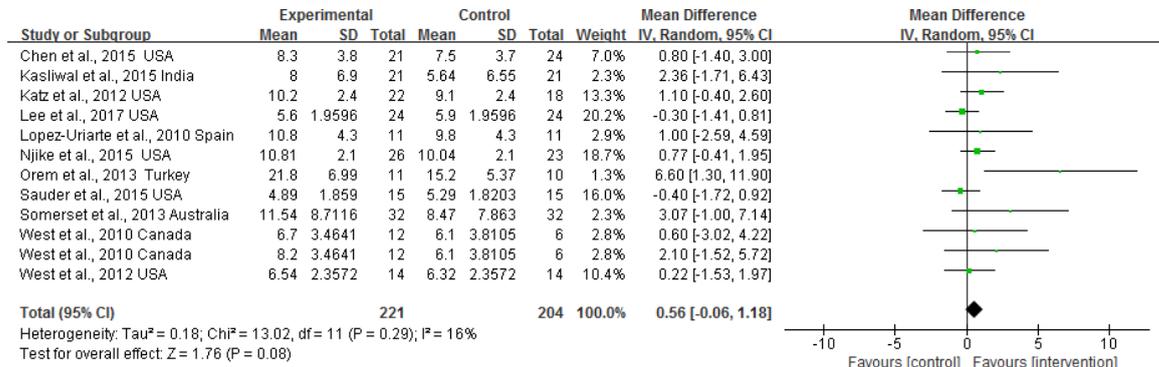


Figure B3. Meta-analysis of studies consuming nuts on adiponectin (ug/ml)

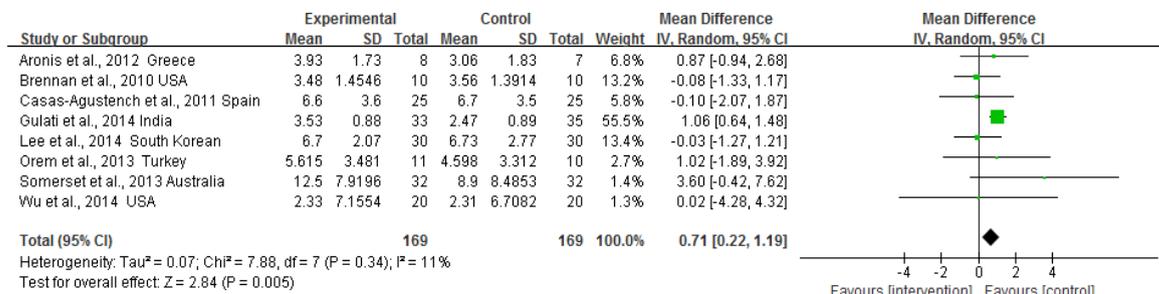


Figure B4. Meta-analysis of studies consuming nuts on oxidized-LDL (mg/dl).

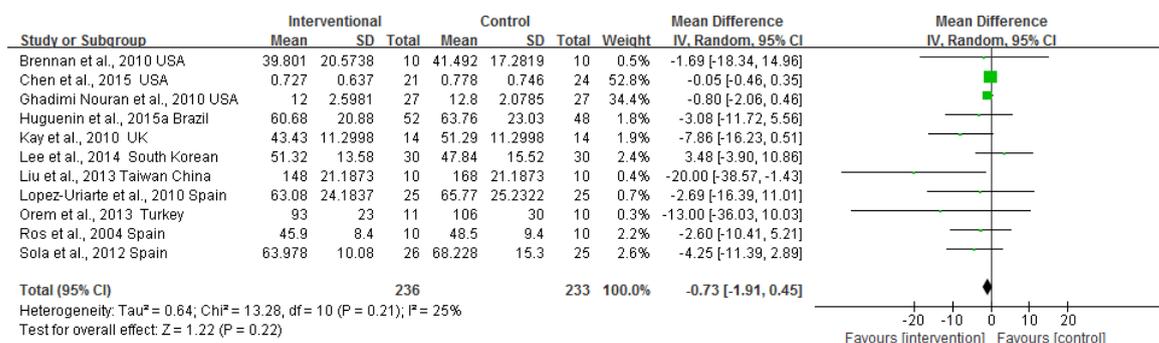


Figure B5. Meta-analysis of studies consuming nuts on Lag time of LDL.

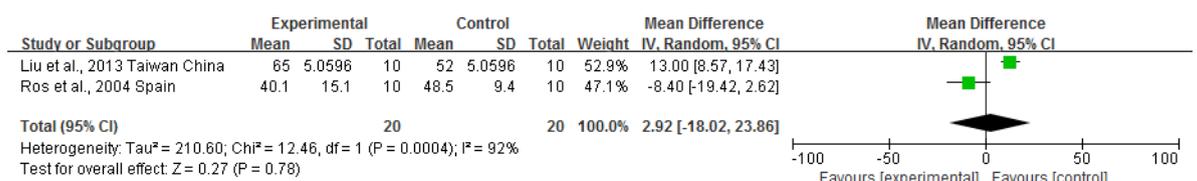


Figure B6. Meta-analysis of studies consuming nuts on SBP (mmHg).

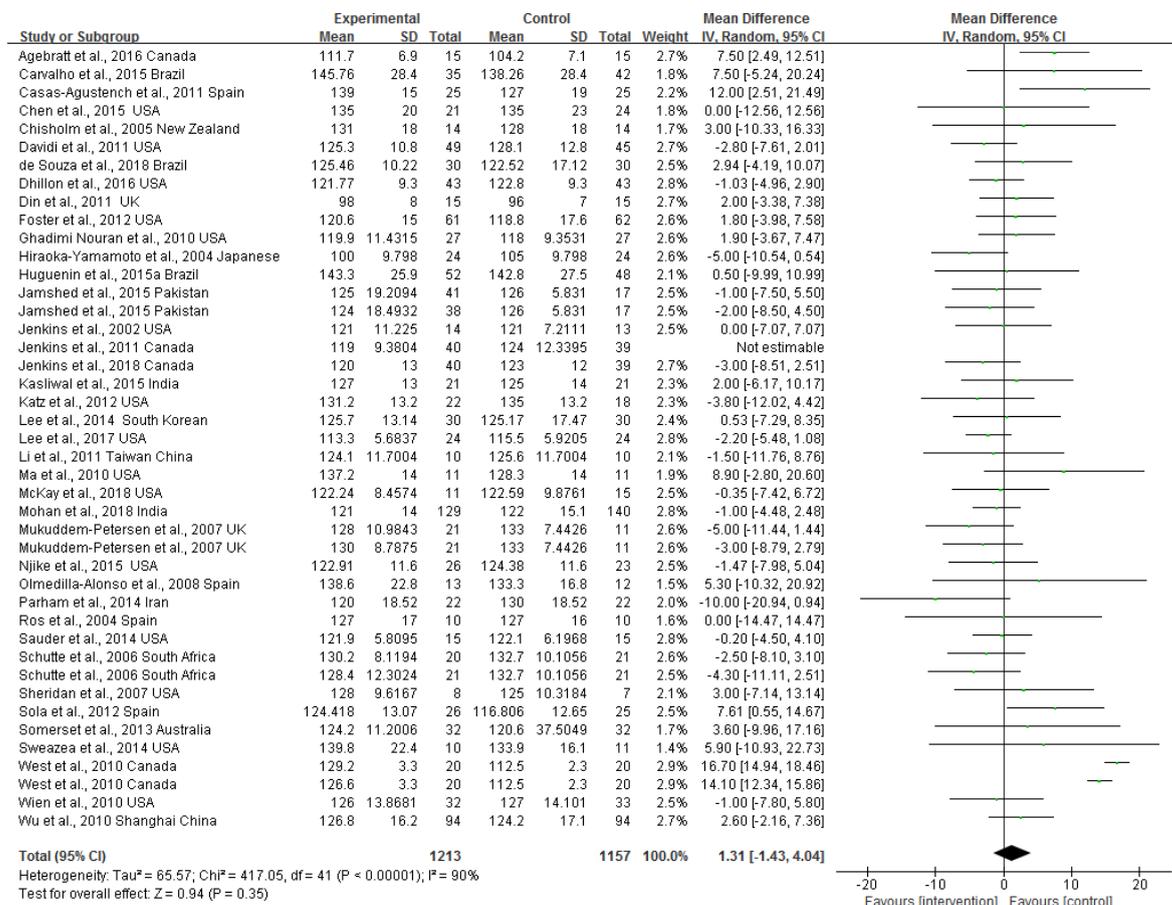


Figure B7. Meta-analysis of studies consuming nuts on DBP (mmHg).

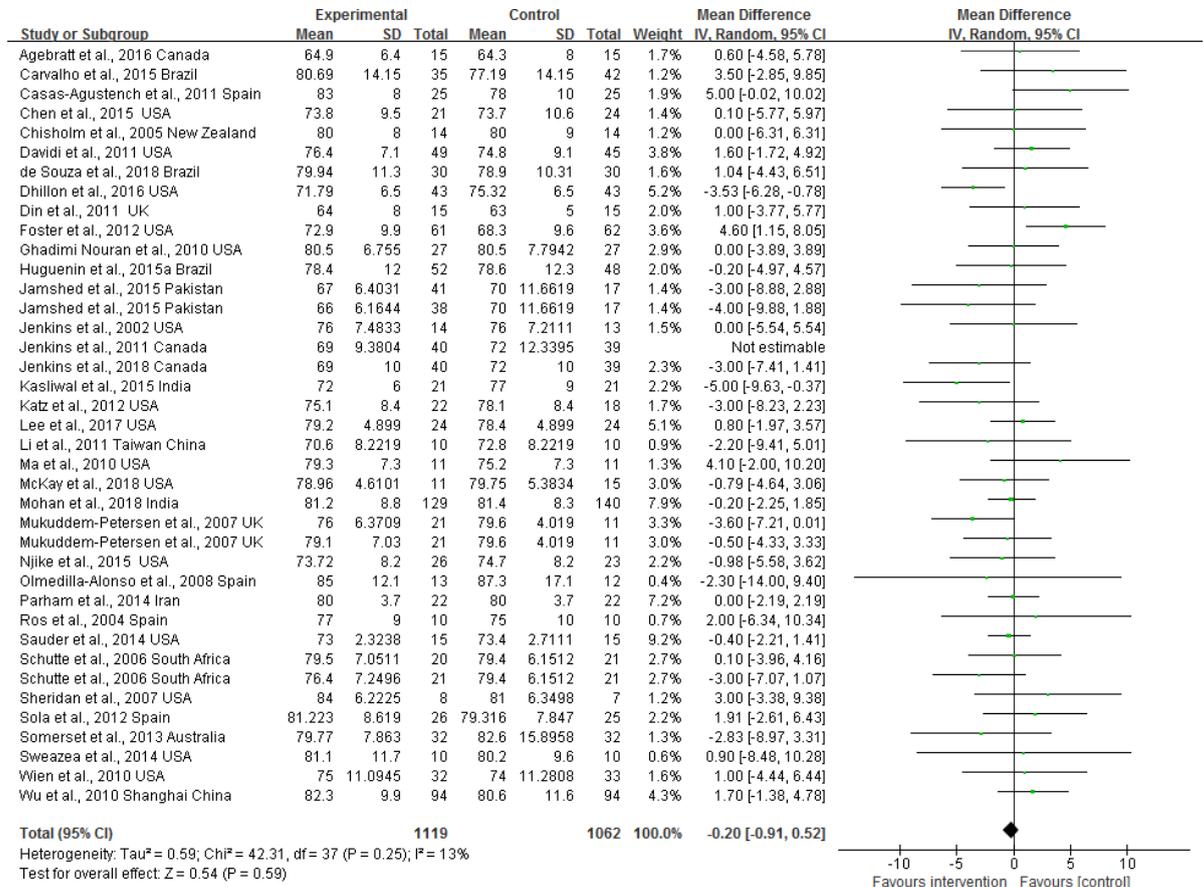


Figure B8. Meta-analysis of studies consuming nuts on Pulse BP (mmHg).

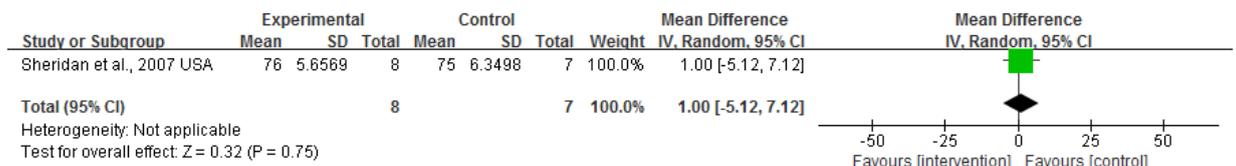


Figure B9. Meta-analysis of studies consuming nuts on TAC (U/mL).

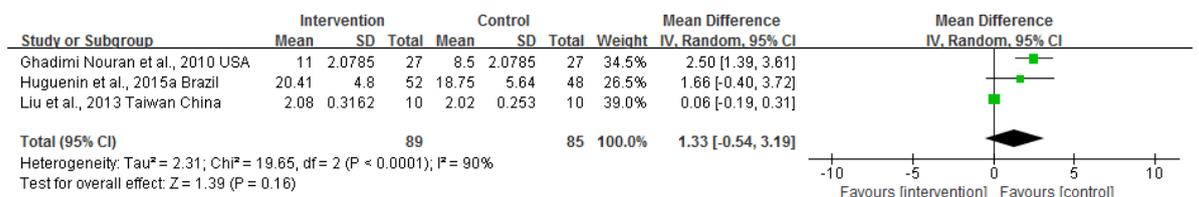


Figure B10. Meta-analysis of studies consuming nuts on atherogenic index of plasma (AIP).

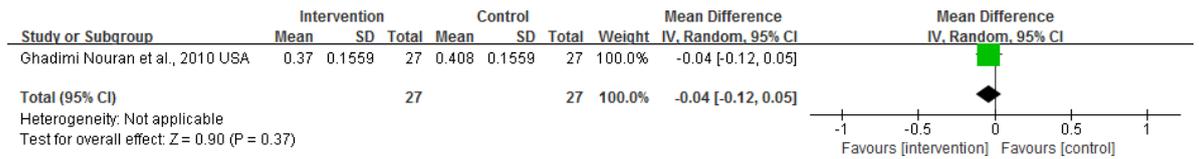


Figure B11. Meta-analysis of studies consuming nuts on ApoA-1 (mg/dl).

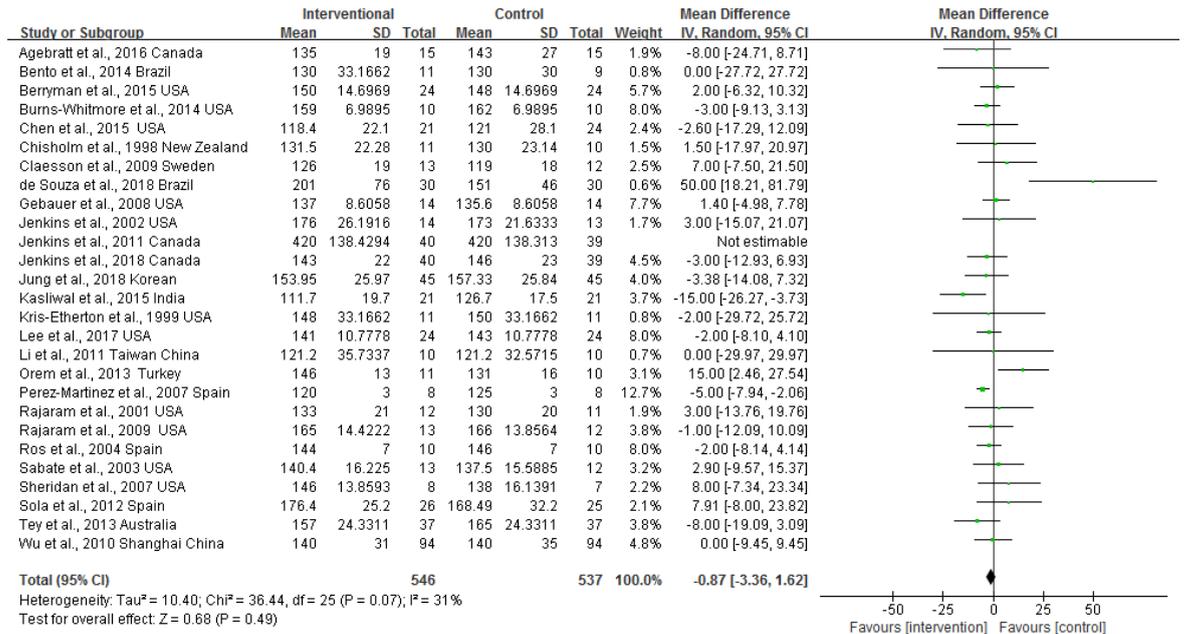


Figure B12. Meta-analysis of studies consuming nuts on nonesterified Fatty acids (mmol/L).

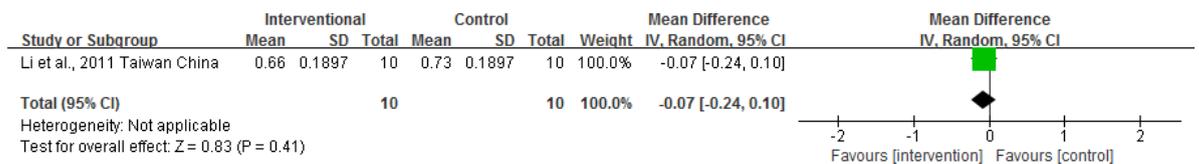


Figure B13. Meta-analysis of studies consuming nuts on hs-CRP (pg/mL).

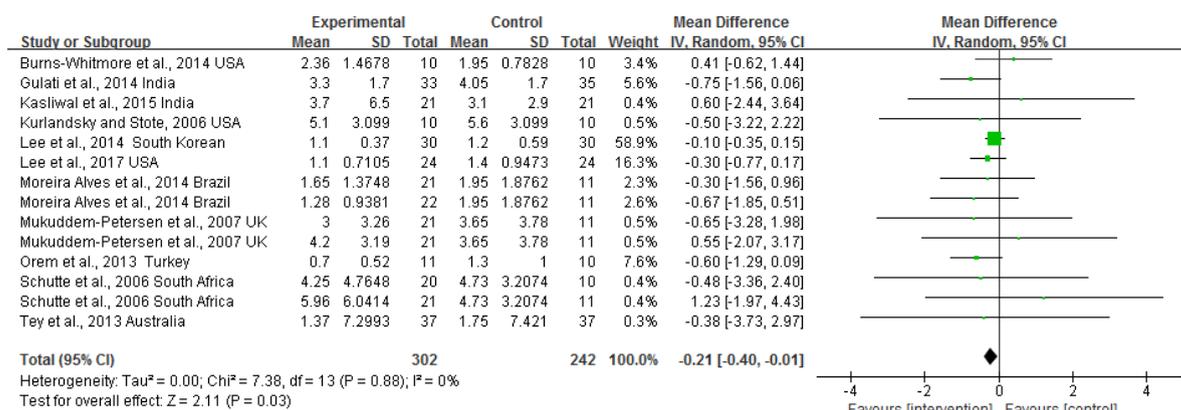


Figure B14. Meta-analysis of studies consuming nuts on CRP (ug/mL).

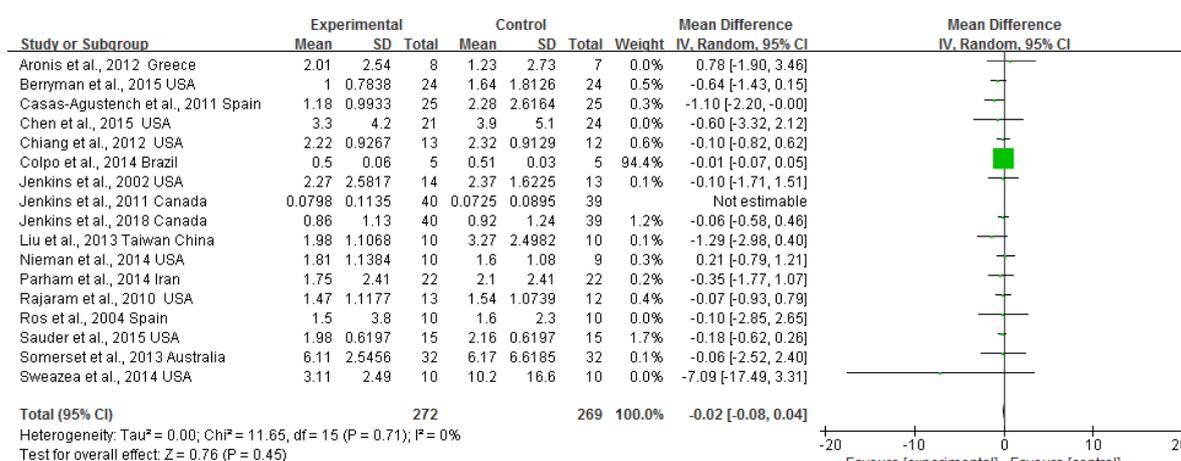


Figure B15. Meta-analysis of studies consuming nuts on TNF-a (pg/mL).

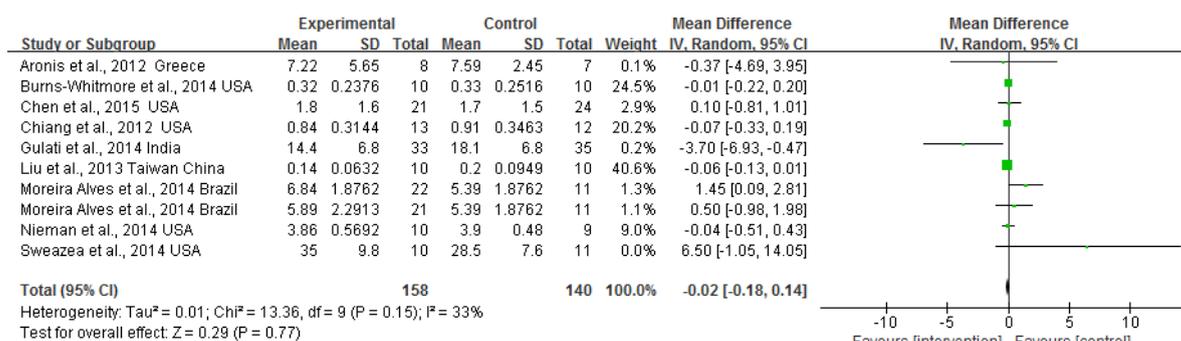


Figure B16. Meta-analysis of studies consuming nuts on IL-10 (pg/mL).

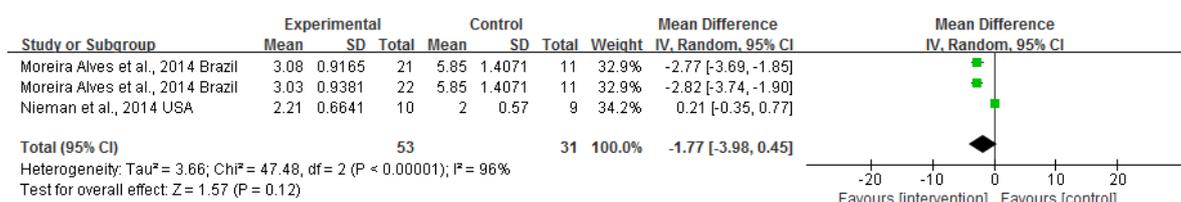


Figure B17. Meta-analysis of studies consuming nuts on IL-8 (pg/mL).

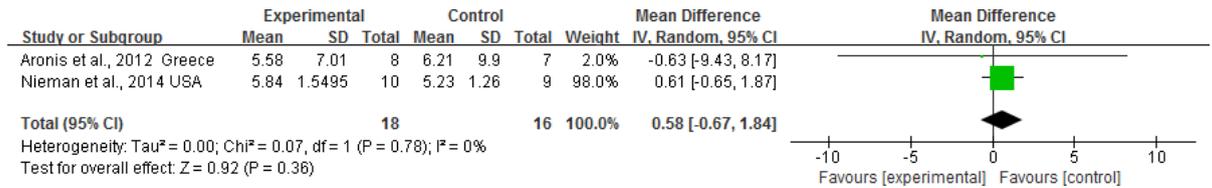


Figure B18. Meta-analysis of studies consuming nuts on IL-6 (pg/mL).

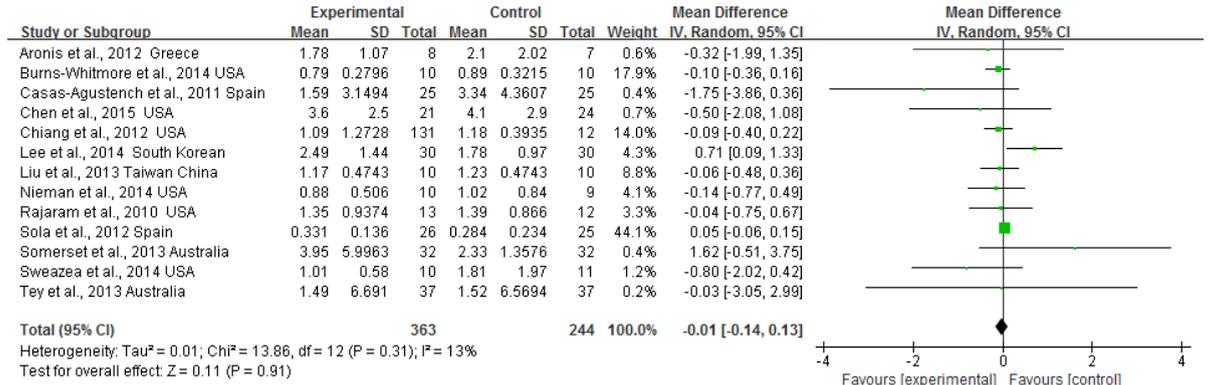


Figure B19. Meta-analysis of studies consuming nuts on IL-1 (pg/mL).

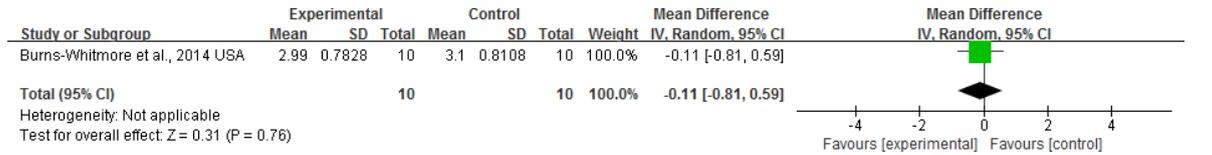


Figure B20. Meta-analysis of studies consuming nuts on IL-18 (pg/mL).

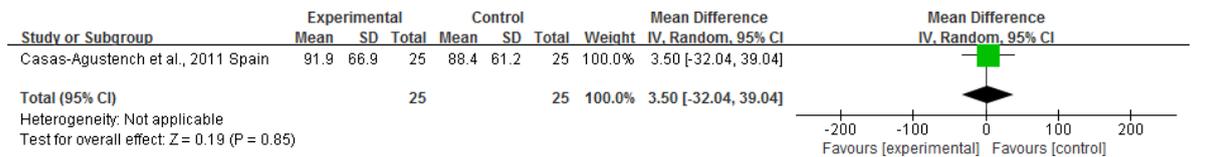


Figure B21. Meta-analysis of studies consuming nuts on IL-1B (pg/mL).

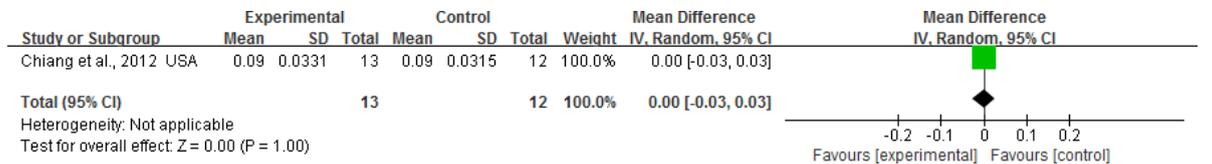


Figure B22. Meta-analysis of studies consuming nuts on sICAM-1 (ng/mL).

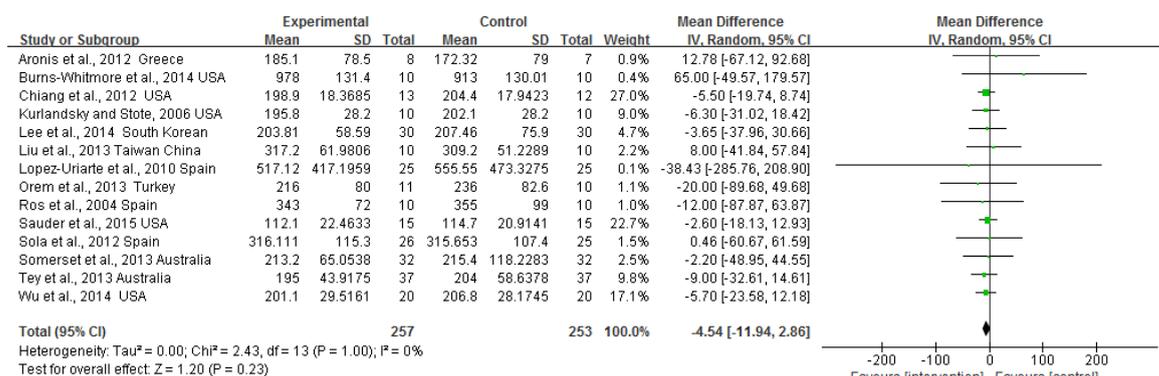


Figure B23. Meta-analysis of studies consuming nuts on sVCAM-1 (ng/mL).

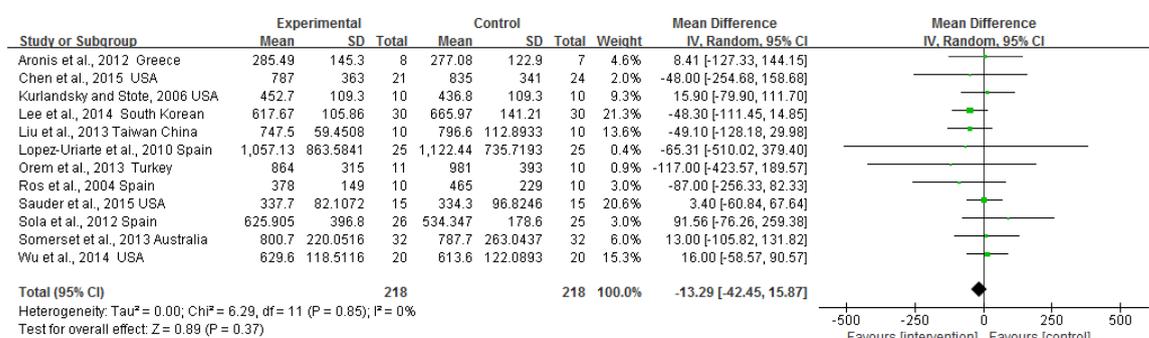


Figure B24. Meta-analysis of studies consuming nuts on sICAM-3 (ng/mL).

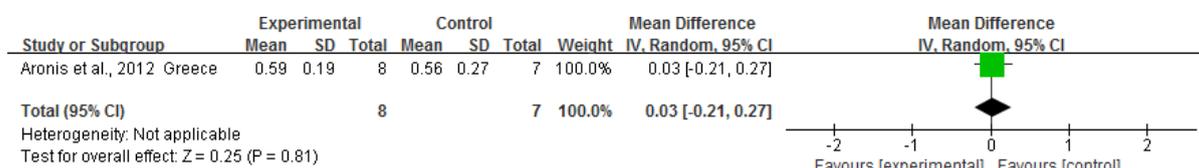


Figure B25. Meta-analysis of studies consuming nuts on SAA (ug/mL).

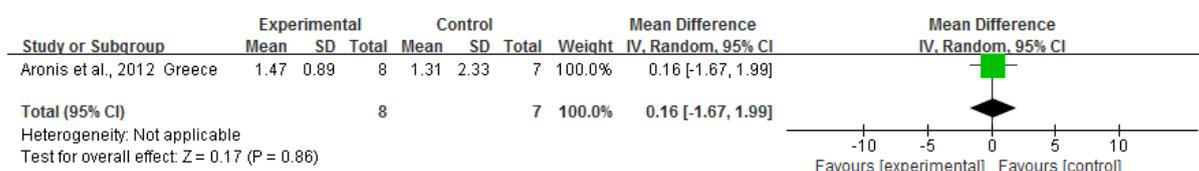


Figure B26. Meta-analysis of studies consuming nuts on plasma E-Selectin (ng/mL).

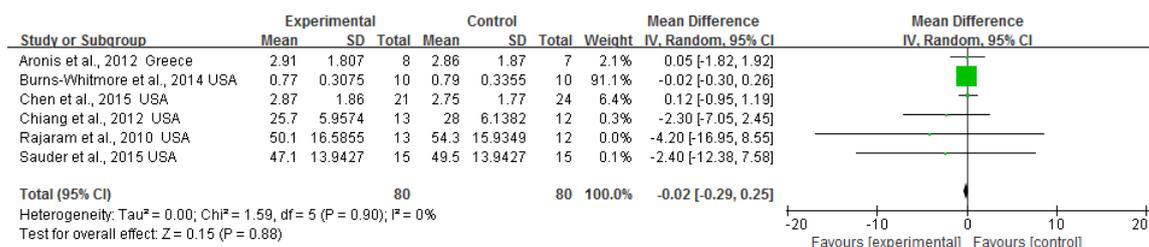


Figure B27. Meta-analysis of studies consuming nuts on P-Selectin (ng/mL).

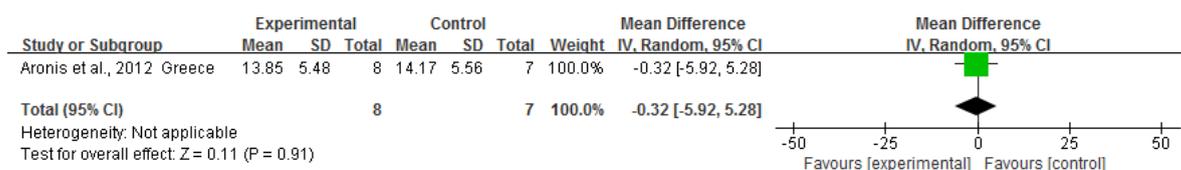


Figure B28. Meta-analysis of studies consuming nuts on Thrombomodulin-1 (ng/mL).

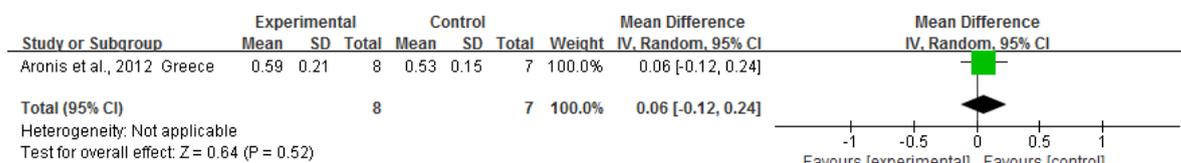


Figure B29. Meta-analysis of studies consuming nuts on PAI-1 (ng/mL).

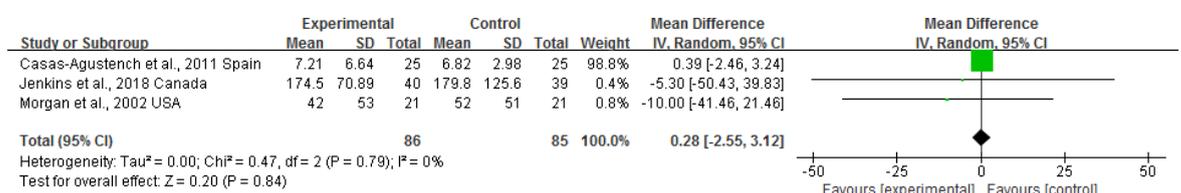


Figure B30. Meta-analysis of studies consuming nuts on MCP-1 (pg/mL).

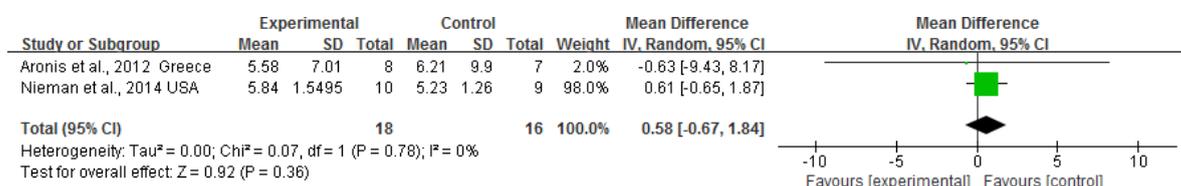


Figure B31. Meta-analysis of studies consuming nuts on PWV (m/s).

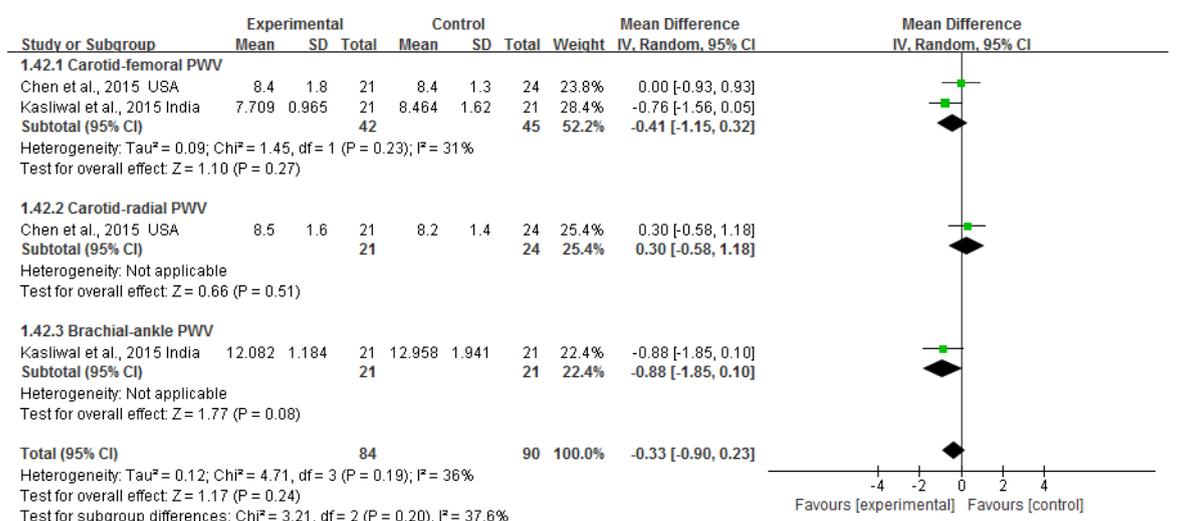


Figure B32. Meta-analysis of studies consuming nuts on Hyperemic blood flow (mL/min).

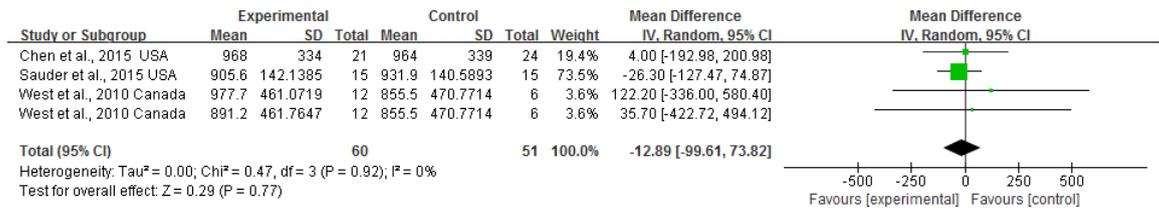


Figure B33. Meta-analysis of studies consuming nuts on Post- velocity (cm/s).

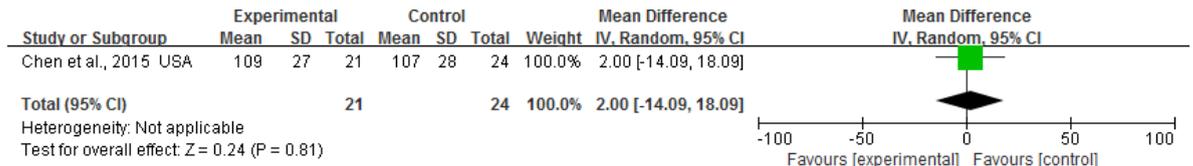


Figure B34. Meta-analysis of studies consuming nuts on plasma nitric oxide (NO) (umol/L).

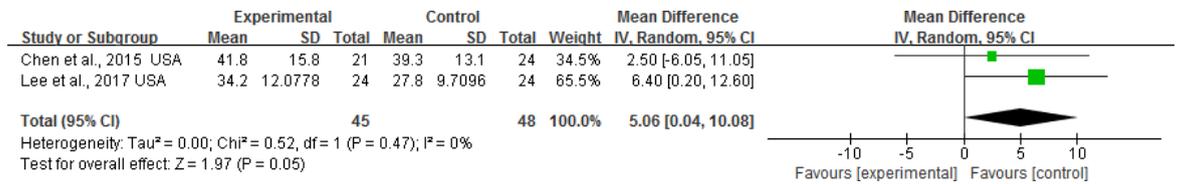


Figure B35. Meta-analysis of studies consuming nuts on TBARS (uM).

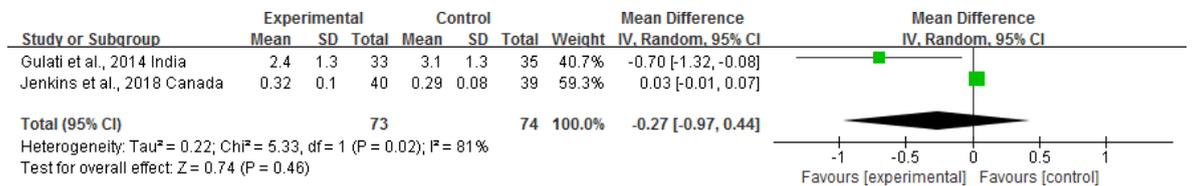


Figure B36. Meta-analysis of studies consuming nuts on Atherogenic Index (AI).

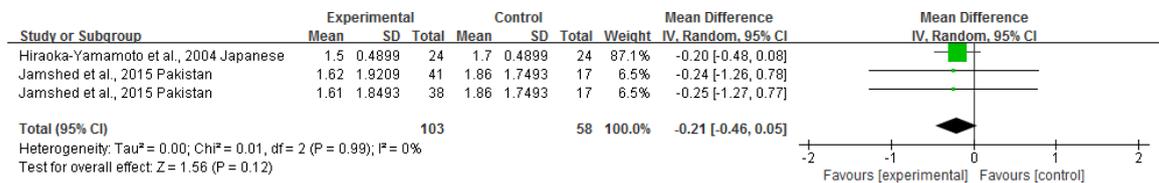


Figure B37. Meta-analysis of studies consuming nuts on tissue plasminogen activator (TPA) (ug/L).

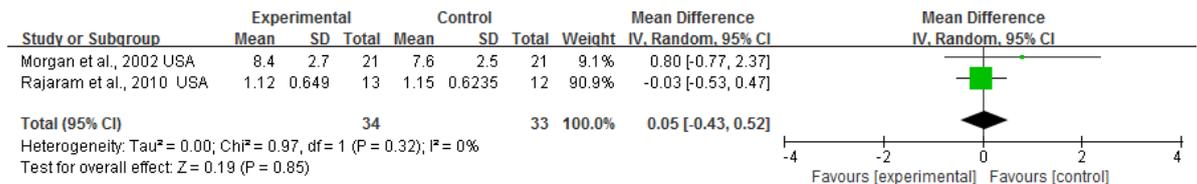


Figure B38. Meta-analysis of studies consuming nuts on Fibrinogen (g/L).

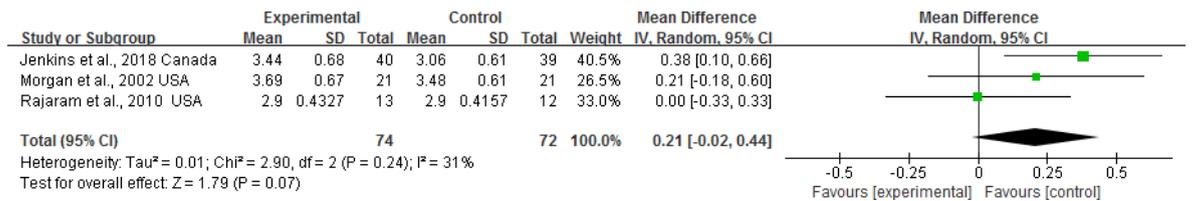


Figure B39. Meta-analysis of studies consuming nuts on Endothelial-1 (fmol/ml).

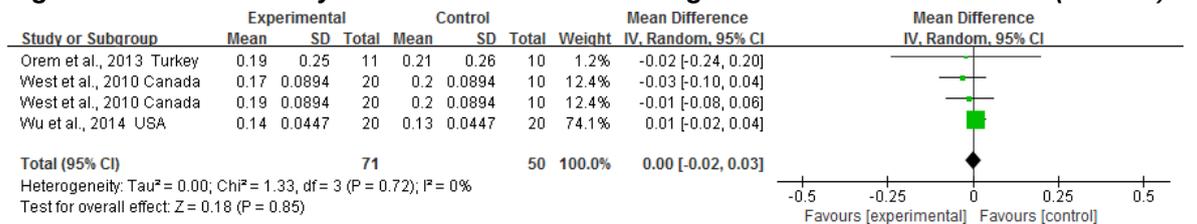


Figure B40. Meta-analysis of studies consuming nuts on endothelium-independent vasodilation (EIV) (%).

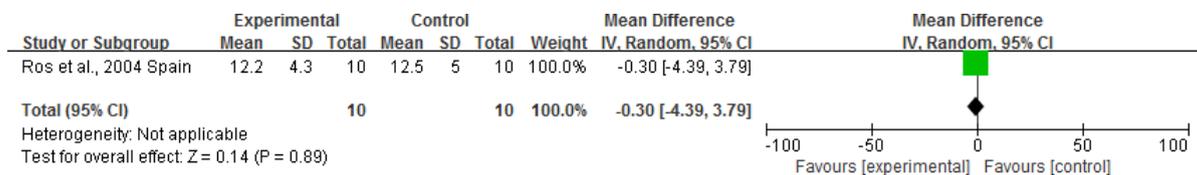


Figure B41. Meta-analysis of studies consuming nuts on endothelium-dependent vasodilation (EDV) (%).

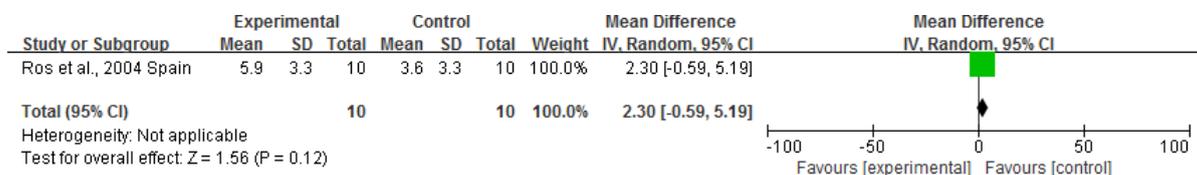


Figure B42. Meta-analysis of studies consuming nuts on RHI.

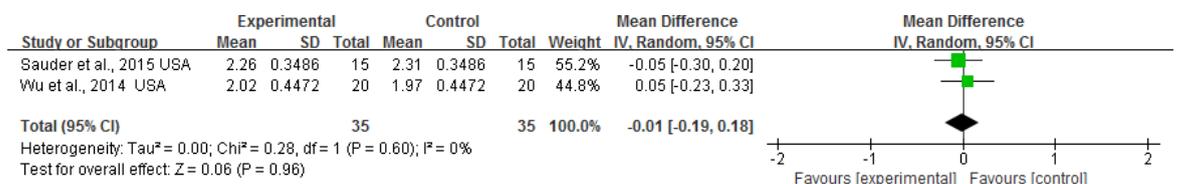


Figure B43. Meta-analysis of studies consuming nuts on Augmentation Index (AI).

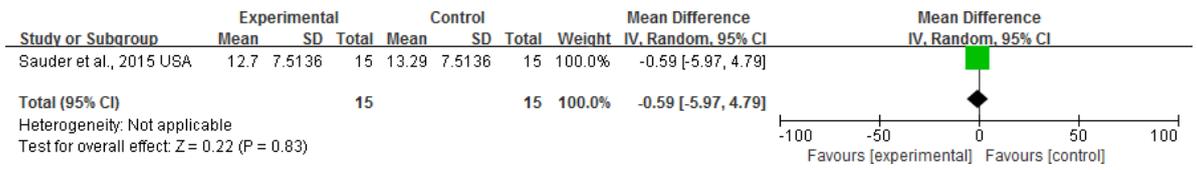
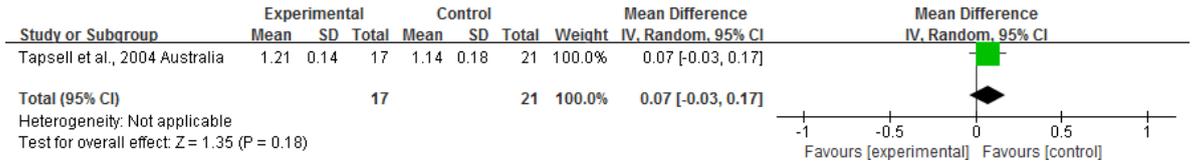


Figure B44. Meta-analysis of studies consuming nuts on total antioxidant status (TAS) (mmol/L).



Appendix B45. Nutrition Society Student Conference 2016 presentation

Dear Author,

Thank you for submitting your Original Communication(s) for consideration by the selection panel for presentation at the Nutrition Society Student Conference 2016.

I am delighted to tell you that your Original Communication (OC) has been accepted for **lightening presentation** at the meeting. Attached to this message you will find the running order. Please look carefully through the document to find your OC. We have done our very best to accommodate preferences for Oral, Poster and Lightening presentations although this has not always been possible - as ever we have tried to produce a balanced programme which will be of interest to the delegates. **The running order highlights if you will be presenting a poster, oral or lightening session. Please ensure to check the presentation format and follow the appropriate instructions below.**

The University of Chester uses PowerPoint 2010 and facilities will be available for standard PowerPoint presentations using PC format.

Save presentations in this format and email to Barbara at 1324743@chester.ac.uk

A back-up copy should be saved onto a memory stick and brought to the meeting.

Finally, authors are reminded that they **MUST** register to attend the meeting. To register for the conference, please see: <https://www.eventbrite.co.uk/e/student-conference-2016-tickets-25717026318?ref=ebtn>

[Student Conference 2016 Tickets, Thu, 8 Sep 2016 at 09:00 ...](#)

www.eventbrite.co.uk

Eventbrite - The Nutrition Society presents Student Conference 2016 - Thursday, 8 September 2016 | Friday, 9 September 2016 at Riverside Innovation Centre.

I very much look forward to seeing you at the meeting in Chester.

If you have any queries, please contact: Tokunbo Osho (adetokunboosho@yahoo.com)

Kind Regards,
Barbara Bray

Efficacy of nutritional interventions supplementing nuts on cardiovascular risk factors among adults individuals: a systematic review and meta-analysis of intervention studies. By F Liang, J Young and J

Lara, Department of Applied Sciences, Faculty of Health and Life Sciences, University of Northumbria at Newcastle, NE18ST, UK



Contact: fan.liang@northumbria.ac.uk

BACKGROUND

- Current evidence suggests the prevalence of healthy ageing is relatively low (1). Ageing is characterised by the degree to which body functions decline, including declines in cardiovascular function (2).
- As CVDs remain the leading cause of mortality in Europe (3), epidemiological evidence reported an positive association between nuts intake and lower risk for CVDs.
- No large-scale SR studied on whether factors - ethnicity plays a role in this association or not.

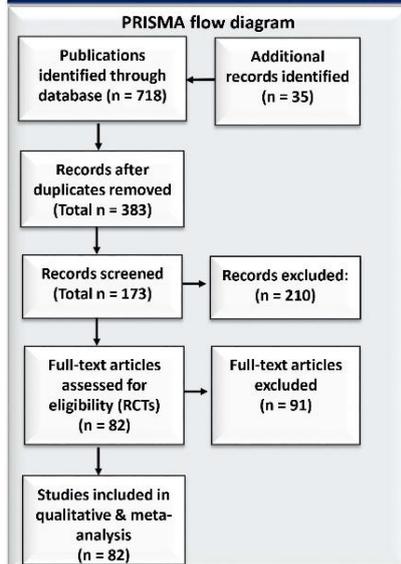
OBJECTIVE

- To critically evaluate the evidence on the effect of supplementing nuts on CV risk factors in Asian and non-Asian populations.

METHOD

- This systematic review was undertaken in adherence to Cochrane guidance. The protocol is registered at PROSPERO (CRD42018089055).
- Terms were utilize in search strategy (MEDLINE; Scopus; Web of Science): tree nuts/groundnuts, RCTs, CVDs, measurements for arterial stiffness, FMD, blood lipids, inflammatory biomarkers, pro-inflammatory cytokines, anti-inflammatory cytokines, chemokines and oxidative stress biomarkers.
- Inclusion: Interventions with evaluating the use of nuts; RCTs; adults
- Exclusion: non-cardiovascular outcome measures; non-intervention studies without nuts

RESULTS



Non-Asian population tend to achieve a better results in most blood lipids markers; inflammatory cytokines -adiponectin, hs-CRP, IL-6 and inflammatory biomarkers - sICAM-1 although there is no statistically significant difference.

STRENGTH & LIMITATION

- Strength:**
- Generalizability
 - Low heterogeneity
 - High compliance
- Limitation:**
- Inadequate Asian studies

REFERENCES

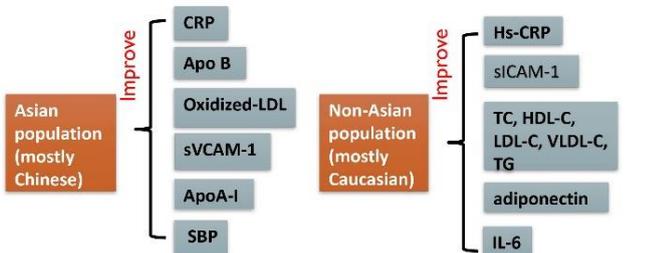
- Hank K. J Gerontol B Psychol Sci Soc Sci. 2011; 66 (2): 230-6.
- Lara J, et al. Maturitas. 2013; 76(2): 189-99.
- Townsend N, et al. Eur Heart J. 2016; 37(42): 3232-45

Table 1. Pooled estimates of effects size for all markers of positive results after nut consumption are summarized

Outcome Parameter	Mean Difference	95% Confidence Interval	p-Value	No. of Studies	Sample Size	I ² (%)
FFA (mmol/L)	-0.03	-0.05 to -0.01	P< 0.001	4	156	0
TC (mg/dL)	-7.54	-10.2 to -4.89	P< 0.001	66	3021	59
HDL-C(mg/dL)	0.89	0.04 to 1.75	P=0.04	67	3063	53
LDL-C (mg/dL)	-7.21	-9.38 to -5.04	P< 0.001	68	3059	68
TG (mg/dL)	-8.83	-13.12 to -4.53	P< 0.001	65	3028	64
VLDL-C (mg/dL)	-2.25	-3.74 to -0.77	P=0.003	10	520	0
ApoB (mg/dL)	-4.47	-7.01 to -1.94	P< 0.001	23	897	64
FMD (%)	0.74	0.09 to 1.39	P=0.03	10	377	5

Table 2. Subgroup analysis was undertaken based on the participants' country & ethnicity

Outcome parameter	Subgroup	Subgroup division	Mean Difference (95% CI)	No. of Studies	p-Value (I ²)
Oxidized-LDL (mg/dL)	Ethnicity	Asian	-20 (-38.57 to -1.43)	1	P=0.15; (51.7%)
		Non-Asian	-1.69 (-18.34 to 14.96)	1	
	Country	Asian	-6.65 (-29.44 to 16.14)	2	P=0.58; (0%)
		Non-Asian	-0.17 (-0.55 to 0.22)	9	
SBP (mmHg)	Ethnicity	Asian	-4.21 (-9.09 to 0.67)	2	P=0.38; (0%)
		Non-Asian	-1.74 (-4.37 to 0.89)	5	
	Country	Asian	-0.67 (-4.57 to 3.23)	4	P=0.35; (0%)
		Non-Asian	1.71 (-1.47 to 4.88)	30	
ApoB100 (mg/dL)	Ethnicity	Asian	-22.9 (-40.92 to -4.88)	1	P=0.07; (70.4%)
		Non-Asian	-5.77 (-8.82 to -2.73)	6	
	Country	Asian	-9.54 (-32.8 to 13.71)	2	P=0.67; (0%)
		Non-Asian	-4.44 (-7.02 to -1.96)	21	
CRP (ug/mL)	Ethnicity	Asian	-1.29 (-2.98 to 0.4)	1	P=0.19; (42.2%)
		Non-Asian	-0.12 (-0.56 to 0.33)	3	
	Country	Asian	-1.29 (-2.98 to 0.4)	1	P=0.14; (54.9%)
		Non-Asian	0 (-0.04 to 0.03)	15	
sVCAM-1 (ng/mL)	Ethnicity	Asian	-49.1 (-128.18 to 29.98)	1	P=0.23; (31.7%)
		Non-Asian	14.24 (-51.12 to 79.6)	2	
	Country	Asian	-48.61 (-97.96 to 0.74)	2	P=0.08; (66.9%)
		Non-Asian	5.65 (-30.49 to 41.79)	10	



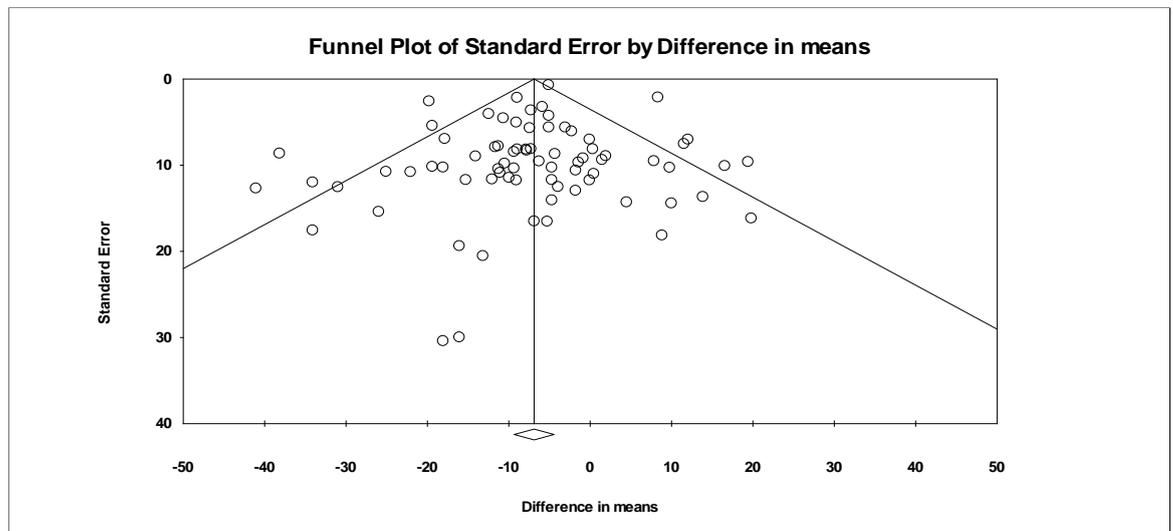
CONCLUSION

- Evidence shows the beneficial effects of general nuts on blood lipids and FMD.
- A valuable tendency of improvement on different cardiovascular markers after nut intake was observed between Asian and non-Asian population.
- These results may be valuable for designing human trials in the near future.
- Large-scale studies should validate such results & more relevant RCTs from Asian population are needed to be researched.

Appendix B46.

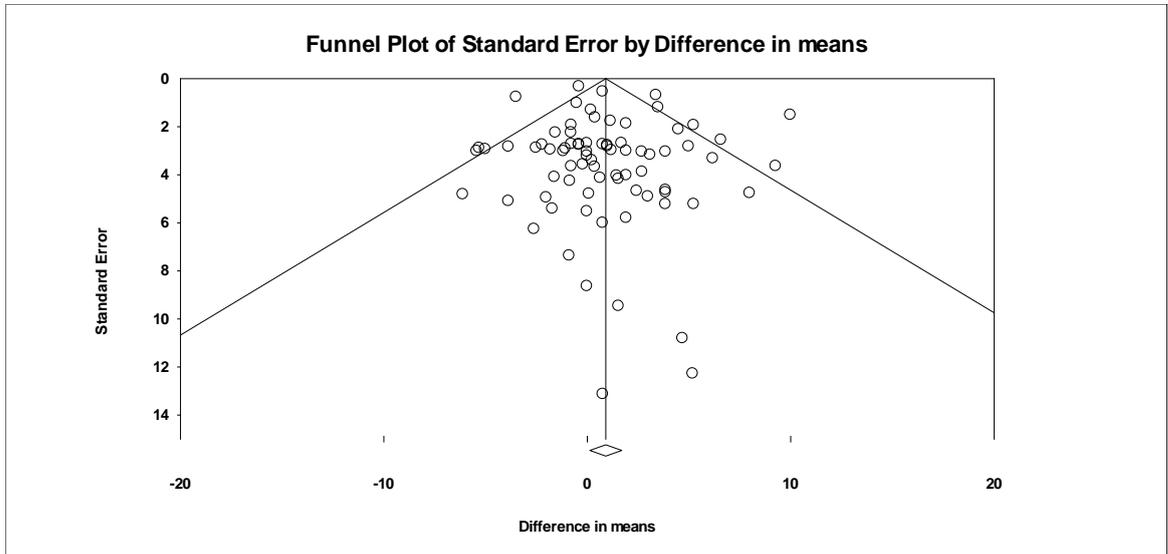
Publication bias assessed by funnel plot of seven parameter outcomes (over or equal to 10 studies included in each outcome) that have positive effects in the meta-analysis. Funnel plot showing study precision against the mean differences effect estimate with 95% confidence intervals (CI) for TC, HDL-C, LDL-C, TG, VLDL-C and Apo B on intervention supplementing nuts. SE = Standard error.

Figure 3.9. Funnel plot of SE for the effect of interventions supplementing nuts on TC (mg/dl) was not completely symmetrical, suggesting that the present study has some slight publication bias. However, the Egger tests ($p=0.28754$) did not give sufficient evidence that the present study had publication bias.



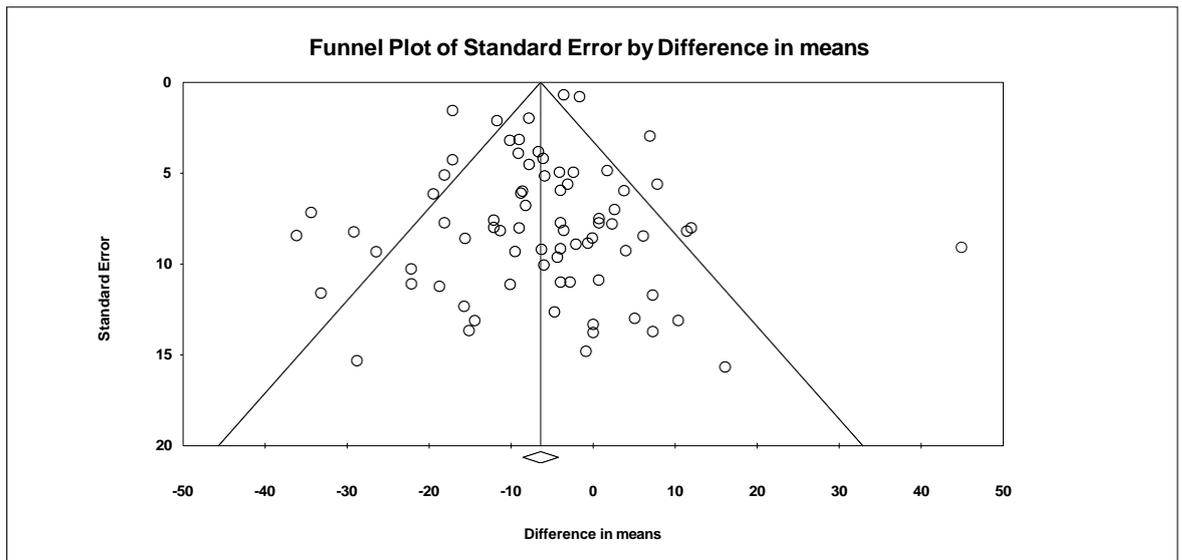
The intercept: -0.25207, 95% CI (-0.72098, 0.21685), with $t=1.07135$, $d.f=73$. The 1-tailed p -value (recommended) is 0.14377, and the 2-tailed p -value is 0.28754.

Figure 3.10. Funnel plot of SE for the effect of interventions supplementing nuts on HDL-C (mg/dl) was not completely symmetrical, suggesting that the present study has some slight publication bias. However, the Egger tests ($p=0.23298$) did not give sufficient evidence that the present study had publication bias.



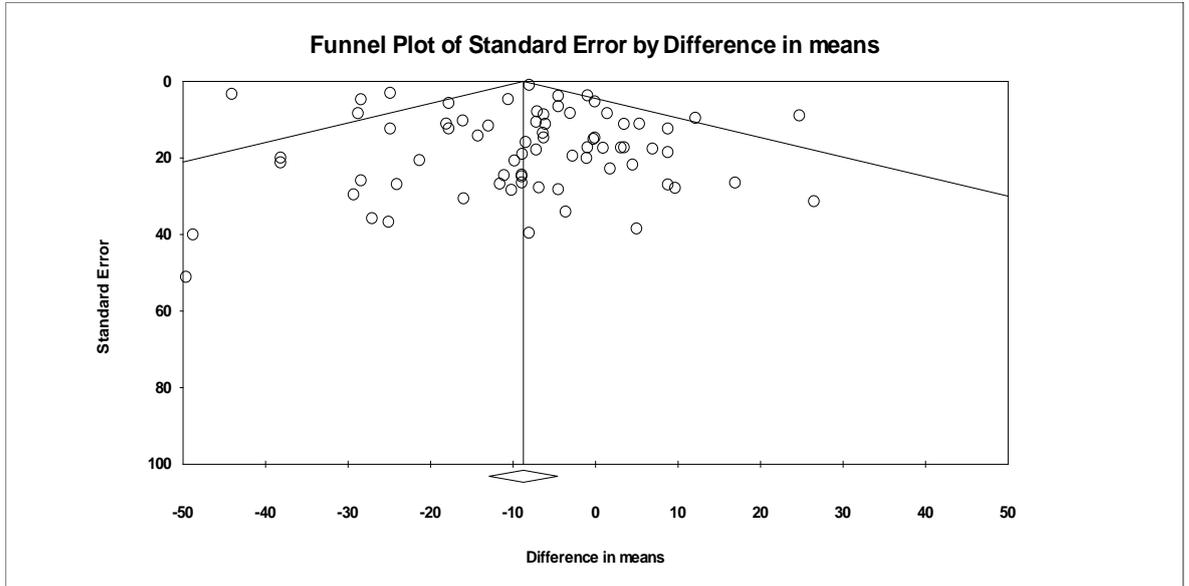
The intercept: 0.27444, 95% CI (-0.18024, 0.72912), with $t=1.20242$, $d.f.=75$. The 1-tailed p -value (recommended) is 0.11649, and the 2-tailed p -value is 0.23298.

Figure 3.11. Funnel plot of SE for the effect of interventions supplementing nuts on LDL-C (mg/dl) was not completely symmetrical, suggesting some slight publication bias. However, the Egger tests ($p=0.23860$) did not give sufficient evidence that the present study had publication bias.



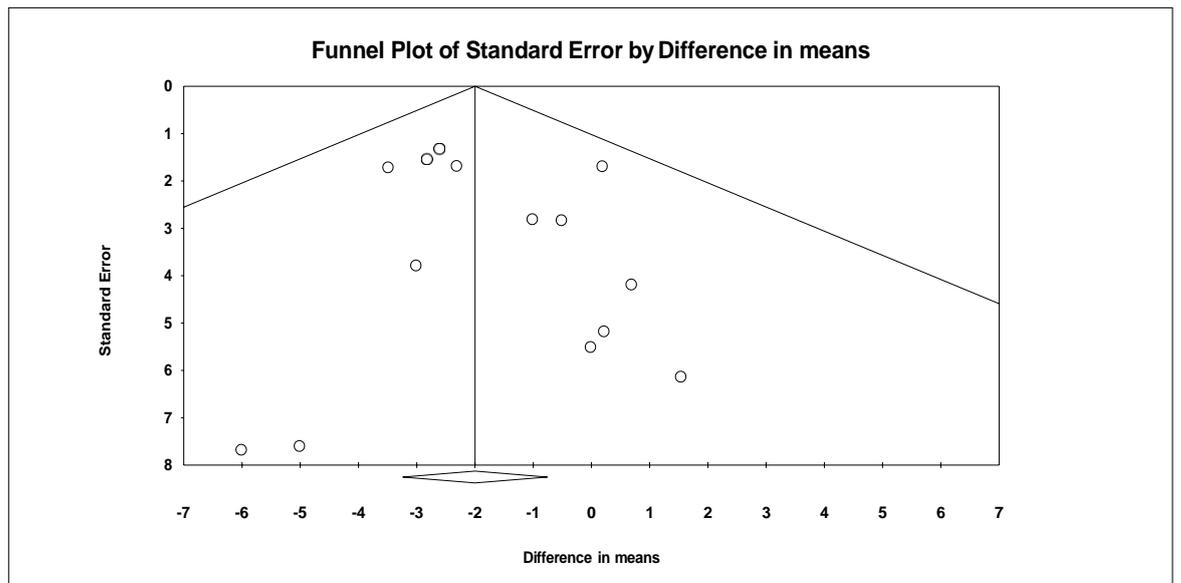
The intercept: -0.33700, 95% CI (-0.90219, 0.22819), with $t=1.18808$, $d.f.=74$. The 1-tailed p -value (recommended) is 0.11930, and the 2-tailed p -value is 0.23860.

Figure 3.12. Funnel plot of SE for the effect of interventions supplementing nuts on TG (mg/dl) was asymmetrical, suggesting this study has publication bias. However, the Egger tests ($p=0.54563$) did not give sufficient evidence that the present study had publication bias.



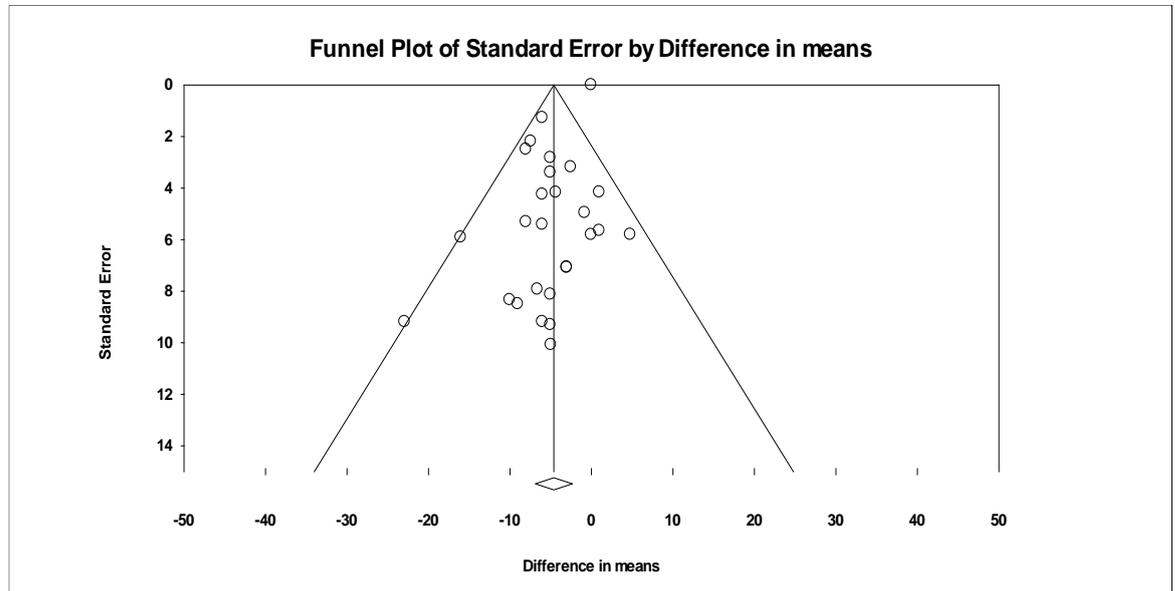
The intercept: 0.15445, 95% CI (-0.35281, 0.66172), with $t=0.60727$, $d.f=70$. The 1-tailed p -value (recommended) is 0.27282, and the 2-tailed p -value is 0.54563.

Figure 3.13. Funnel plot of SE for the effect of interventions supplementing nuts on VLDL-C (mg/dl) was asymmetrical, and statistical analysis using Egger's test ($P=0.39967$), and this revealed that a risk of publication bias was not presented.



The intercept: 0.29150, 95% CI (-0.43581, 1.01880), with $t=0.87325$, $d.f =12$. The 1-tailed p -value (recommended) is 0.19983, and the 2-tailed p -value is 0.39967.

Figure 3.14. Funnel plot of SE for the effect of interventions supplementing nuts on Apo B (mg/dl) was asymmetrical, and statistical analysis using Egger's test ($P=0.00006$), and this revealed that a risk of publication bias was presented.



The intercept: -1.18151 , 95% CI $(-1.68773, -0.67530)$, with $t=4.80699$, $d.f=25$. The 1-tailed p -value (recommended) is 0.00003 , and the 2-tailed p -value is 0.00006 .

Random-effect model – Total cholesterol (TC) regression of length of nuts intervention (days) on difference in means. Difference in means in included studies of TC vs. placebo. The centreline shows the predicted values. Meta-regression analysis was performed by mixed effects regression (unrestricted maximum likelihood) to assess/evaluate the relationship of the cardiovascular biomarkers with difference in means in mixed nuts consumption. Dose of nuts that were not clarified in trials were excluded.

Figure 3.15(a). Meta-regression analysis performed no significant correlations between duration of nuts consumption and TC changes ($P > 0.05$ (0.09958); Slope = 0.01179; $Q = 2.71231$; $d.f. = 1$) in 72 trials.

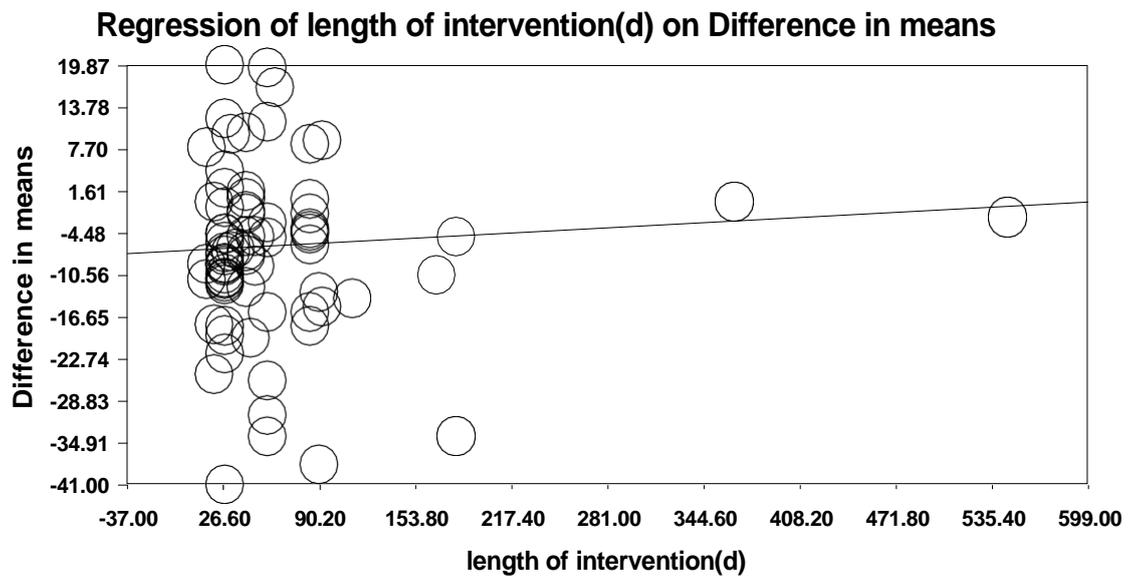


Fig 3.15(b). Meta-regression on the effects of the daily dose of nuts consumption (g) on mean differences in TC indicated that daily dose of nuts consumption may not be associated with lower TC (Slope = 0.00128, $Q = 0.00151$; d.f. = 1; $P > 0.05$ (0.96900)) in 72 studies.

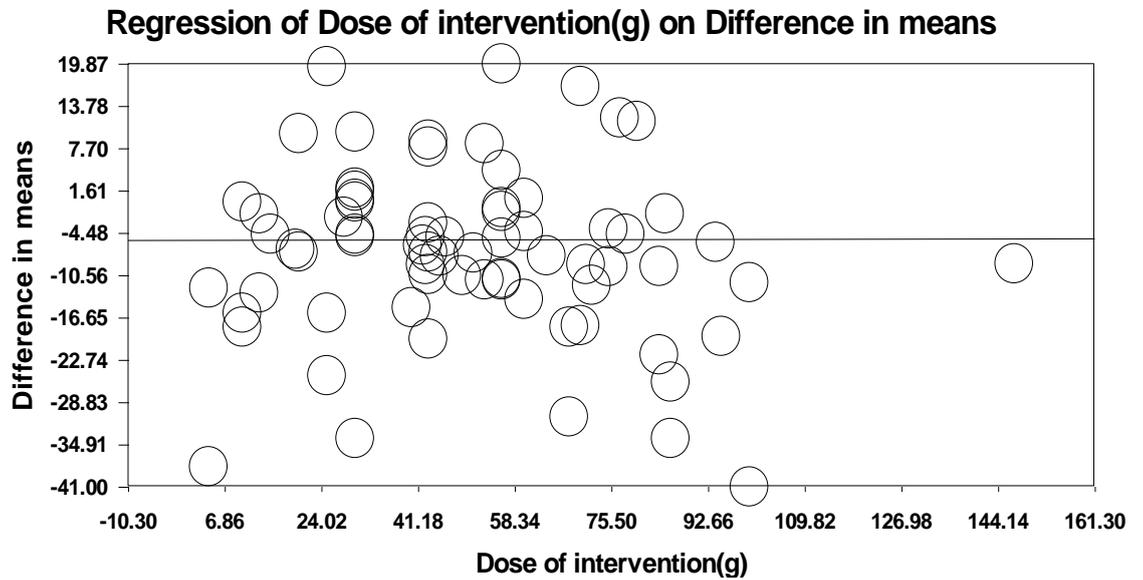


Fig 3.16(a). Meta-regression on the effects of the length of nuts consumption (d) on mean differences in HDL-C indicated that a longer duration of nuts consumption may not be associated with higher HDL-C ($P < 0.05$ (0.00516); Slope = -0.00731; $Q = 7.82178$; d.f. = 1) in 73 studies.

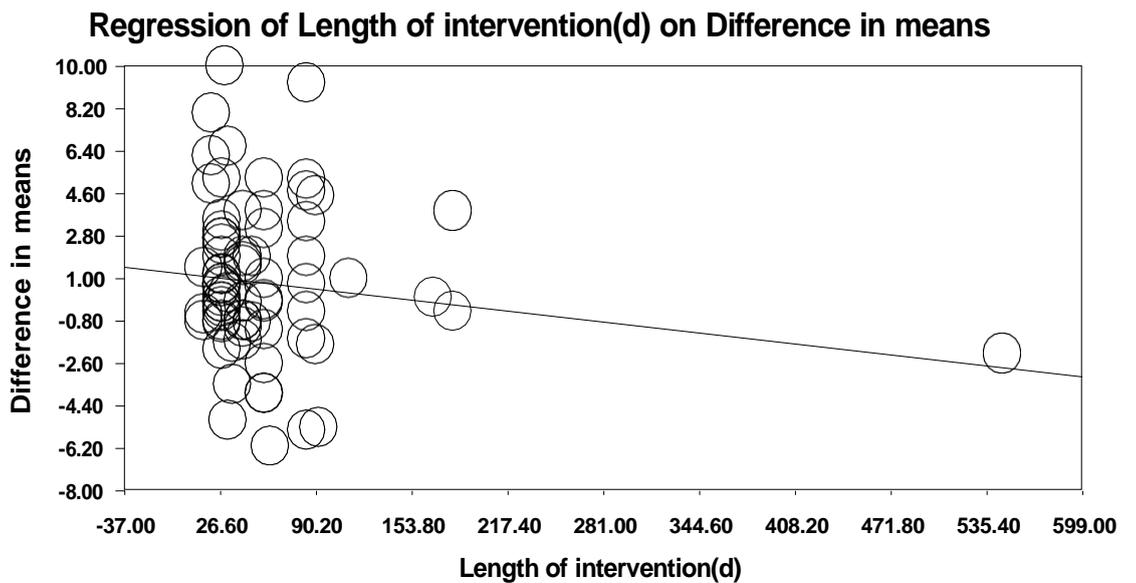


Fig 3.16(b). Meta-regression on the effects of the dose of nuts consumption (g) on mean differences in HDL-C. The meta-regression indicated that the length of nuts consumption may not be associated with difference on HDL-C ($P>0.05$ (0.29533); Slope = 0.00995; $Q = 1.09517$; $d.f = 1$) in 73 trials.

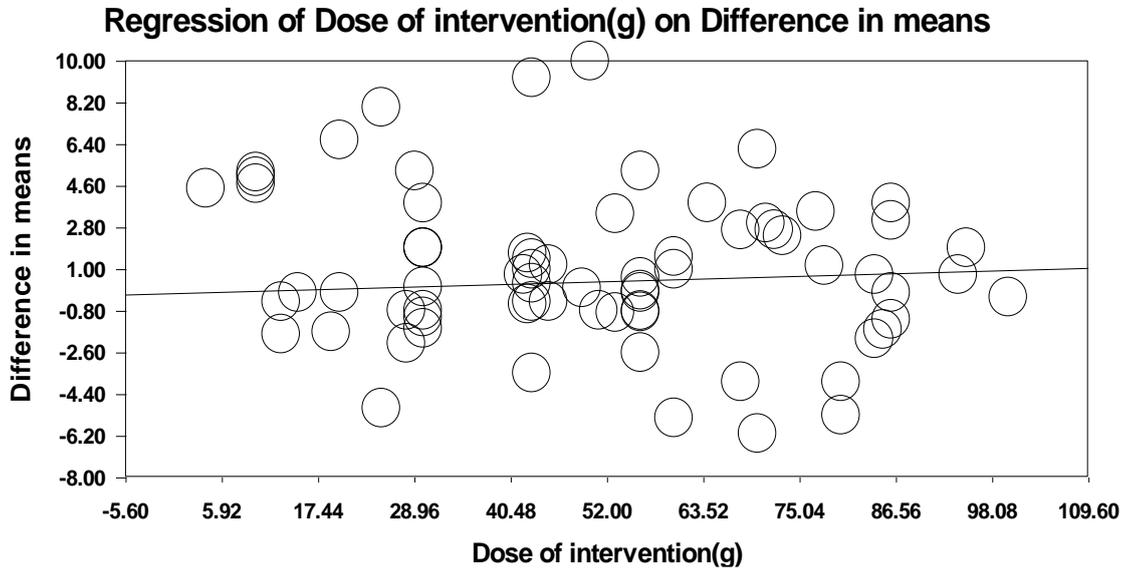


Fig 3.17(a). 74 studies performed meta-regression on the effects of the length of nuts consumption (g) on mean differences in LDL-C. The meta-regression indicated that a longer duration of nuts consumption may be associated with higher LDL-C ($p<0.05$ (0.00996); slope = 0.01358; $q = 6.64190$; $d.f = 1$).

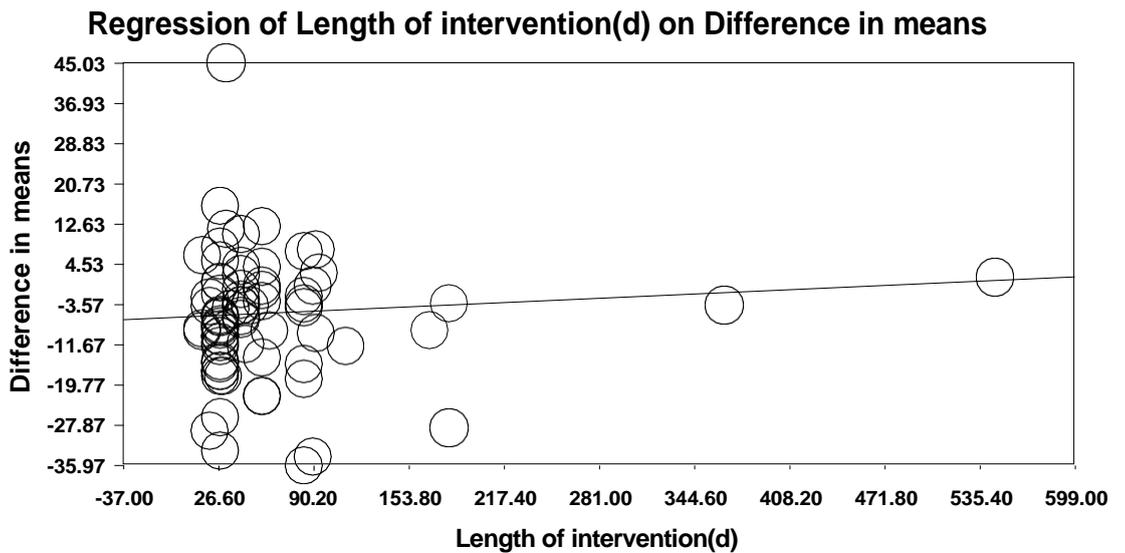


Fig 3.17(b). 74 studies performed meta-regression on the effects of the dose of nuts consumption (g) on mean differences in LDL-C. The meta-regression indicated that a higher amount of nuts consumption may be not associated with lowering LDL-C ($P > 0.05$ (0.50307); $Q = 0.44845$; $d.f = 1$; Slope = -0.01117).

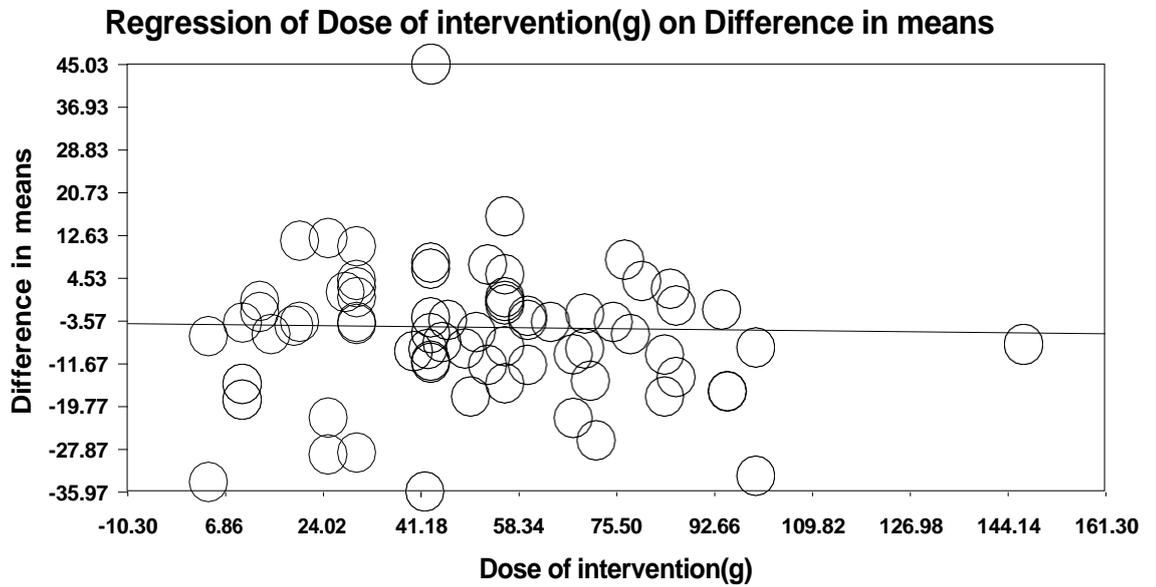


Fig 3.18(a) Meta-regression analysis performed a significant positive correlation between the length of nuts consumption and difference in means of TG and longer duration of nuts intake may be associated with higher TG. ($P < 0.05$ (0.00240); $Q = 9.21674$; Slope = 0.03270; $d.f = 1$) in 71 studies.

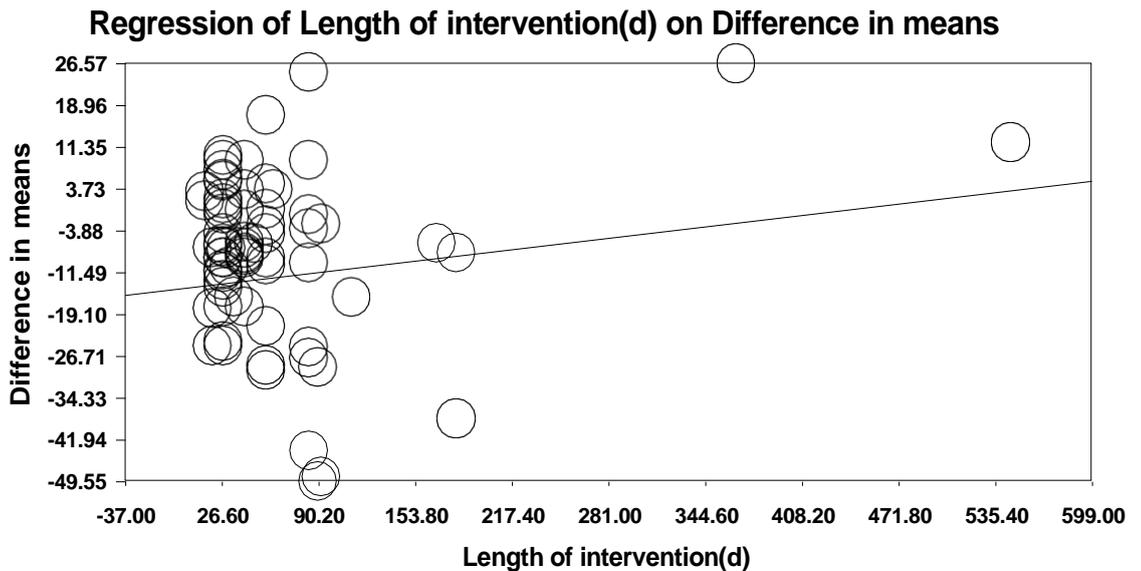


Fig 3.18(b) Meta-regression analysis performed a significant positive correlation between the dose of nuts and difference in means of TG in 71 trials. Greater dose of nut consumption may be associated with lower TG ($P < 0.05$ (0.00392); $Q = 8.32139$; Slope = -0.12699; $d.f = 1$).

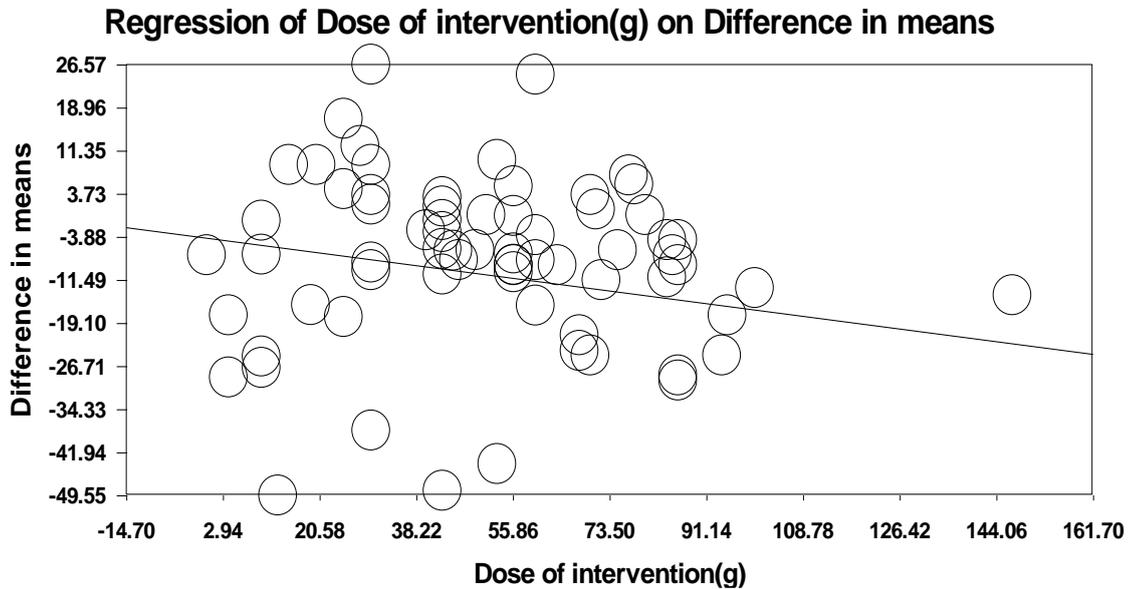


Fig 3.19(a). Meta-regression analysis performed no significant correlations between the length of nuts consumption and difference in means of VLDL-C ($p > 0.05$ (0.64548); $Q = 0.21165$; Slope = 0.00260; $d.f = 1$) in 15 studies.

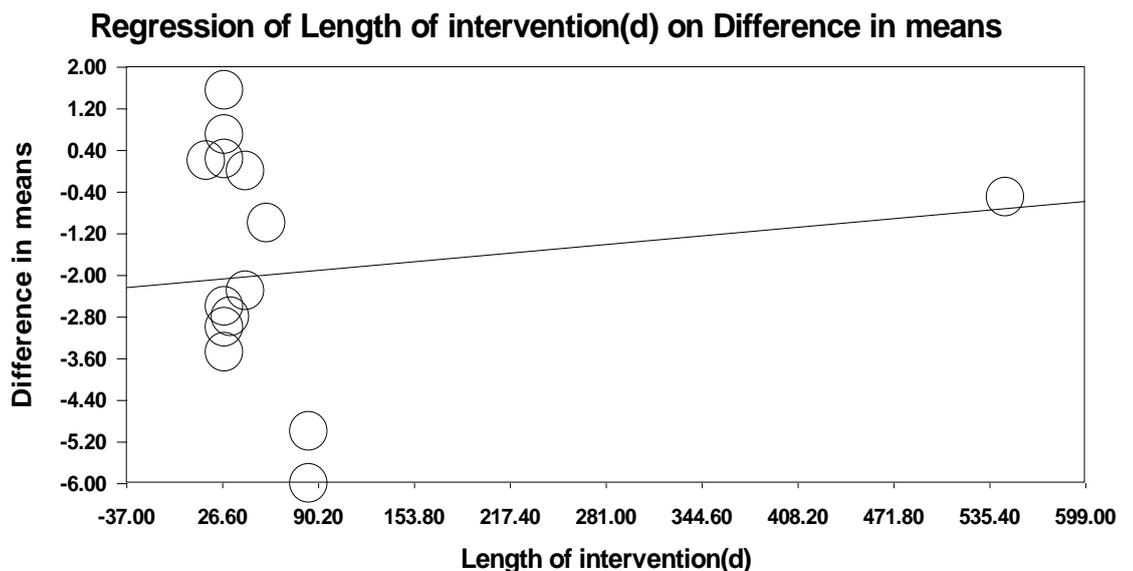


Fig 3.19(b). Meta-regression analysis performed no significant correlations between the daily dose of nuts consumption (g) and difference in means of VLDL-C ($P > 0.05$ (0.32391); $Q = 0.97309$; $d.f = 1$; Slope = -0.02256) in 15 studies.

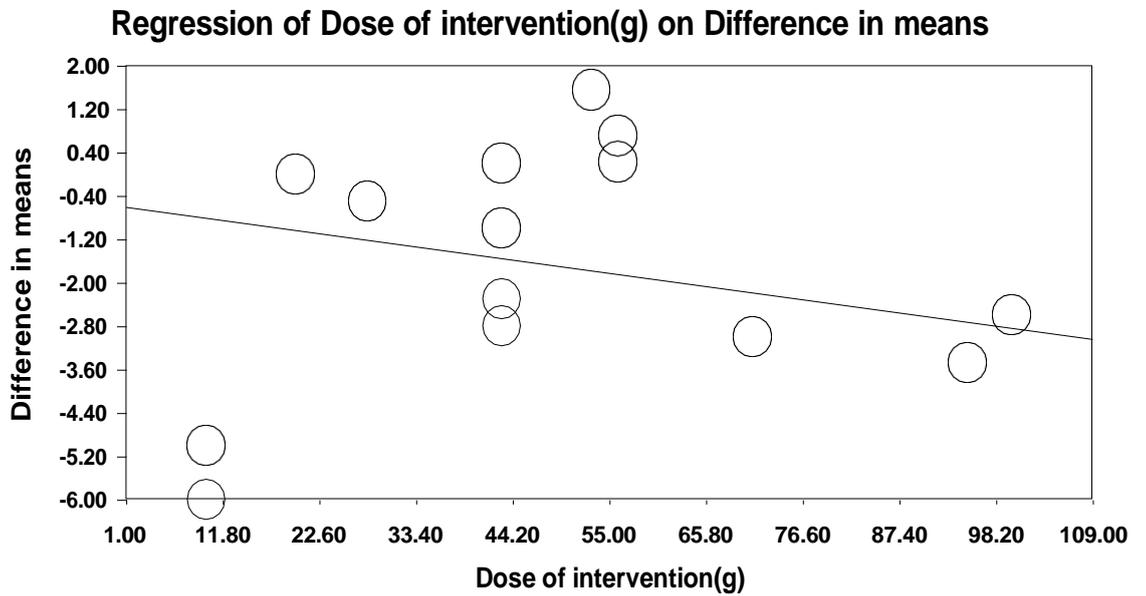


Fig 3.20(a). Meta-regression analysis performed a significant correlation between the length of nuts consumption (days) and difference in means of Apo B ($P < 0.05$ (0.00019); $Q = 13.92838$; $d.f = 1$; Slope = 0.15828) in 27 studies. However, longer duration of nuts intake may not be associated with lower Apo B.

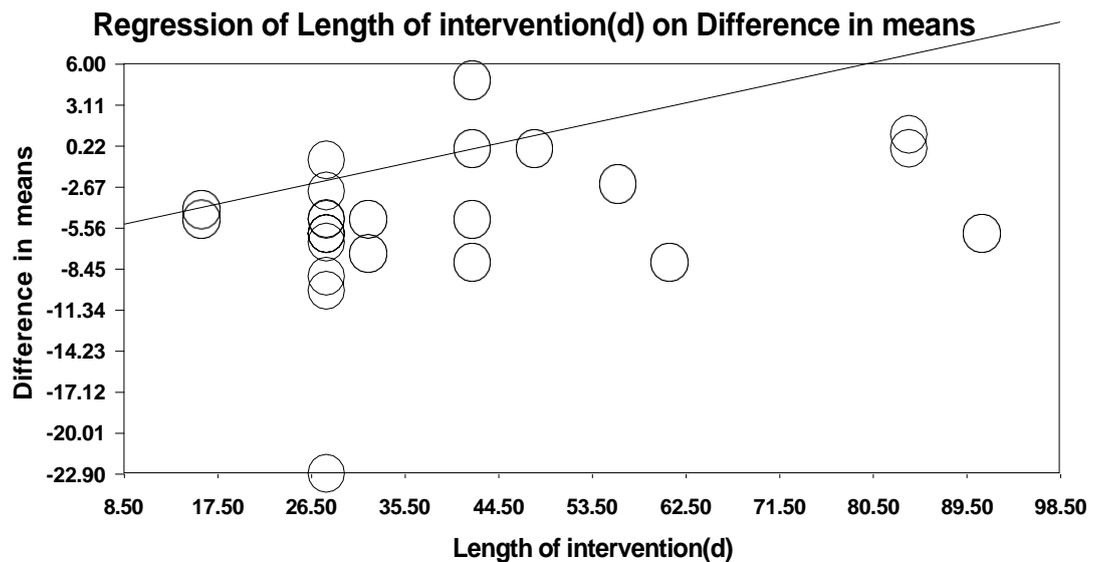
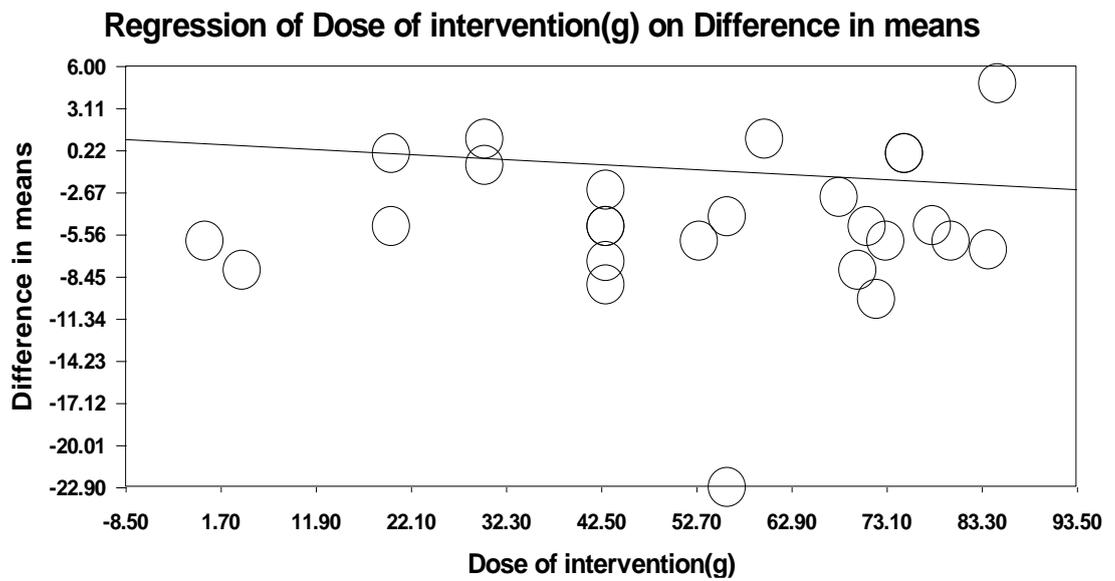


Fig 3.20(b). Meta-regression analysis performed no significant correlations between the daily dose of nuts consumption (g) and difference in means of Apo B ($P > 0.05$ (0.18515); $Q = 0.75581$; $d.f = 1$; Slope = -0.03376) in 27 trials.



Appendix C

Supplementary information for Chapter 4

Table C1: PRISMA Checklist

Section/topic	#	Checklist item	Reported on page #
TITLE			
Title	1	Identify the report as a systematic review, meta-analysis, or both.	140
ABSTRACT			
Structured summary	2	Provide a structured summary including, as applicable: background; objectives; data sources; study eligibility criteria, participants, and interventions; study appraisal and synthesis methods; results; limitations; conclusions and implications of key findings; systematic review registration number.	140
INTRODUCTION			
Rationale	3	Describe the rationale for the review in the context of what is already known.	141 -142
Objectives	4	Provide an explicit statement of questions being addressed with reference to participants, interventions, comparisons, outcomes, and study design (PICOS).	142
METHODS			
Protocol and registration	5	Indicate if a review protocol exists, if and where it can be accessed (e.g., Web address), and, if available, provide registration information including registration number.	142 - 143
Eligibility criteria	6	Specify study characteristics (e.g., PICOS, length of follow-up) and report characteristics (e.g., years considered, language, publication status) used as criteria for eligibility, giving rationale.	143
Information sources	7	Describe all information sources (e.g., databases with dates of coverage, contact with study authors to identify additional studies) in the search and date last searched.	143 -144

Search	8	Present full electronic search strategy for at least one database, including any limits used, such that it could be repeated.	143 -144
Study selection	9	State the process for selecting studies (i.e., screening, eligibility, included in systematic review, and, if applicable, included in the meta-analysis).	143
Data collection process	10	Describe method of data extraction from reports (e.g., piloted forms, independently, in duplicate) and any processes for obtaining and confirming data from investigators.	144 -145
Data items	11	List and define all variables for which data were sought (e.g., PICOS, funding sources) and any assumptions and simplifications made.	145 -146
Risk of bias in individual studies	12	Describe methods used for assessing risk of bias of individual studies (including specification of whether this was done at the study or outcome level), and how this information is to be used in any data synthesis.	147 - 149
Summary measures	13	State the principal summary measures (e.g., risk ratio, difference in means).	149
Synthesis of results	14	Describe the methods of handling data and combining results of studies, if done, including measures of consistency (e.g., I ²) for each meta-analysis.	148 - 150

Figure C2. Meta-analysis of studies consuming olive oil on TC (mmol/L).

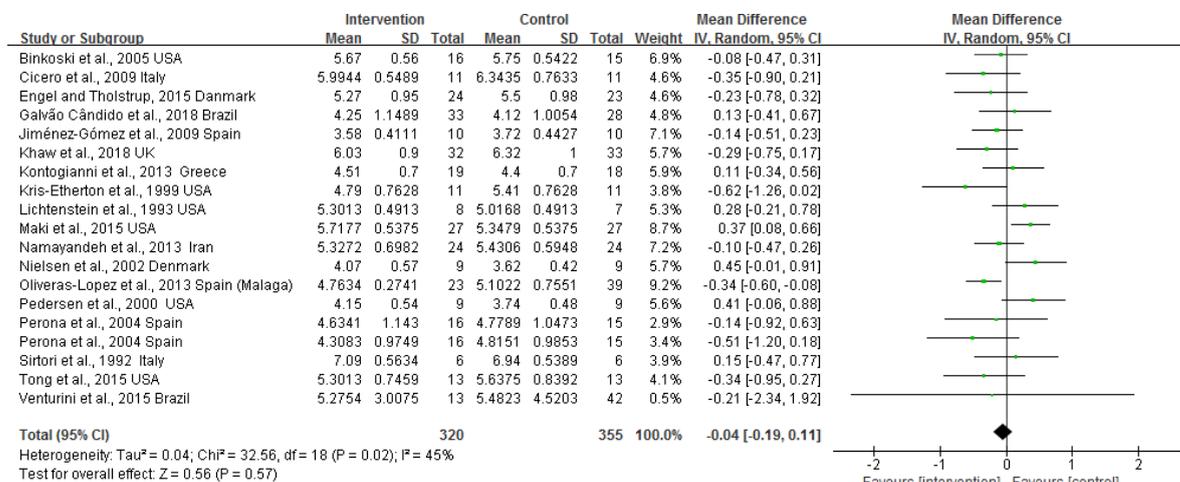


Figure C3. Meta-analysis of studies consuming olive oil on HDL-C (mmol/L).

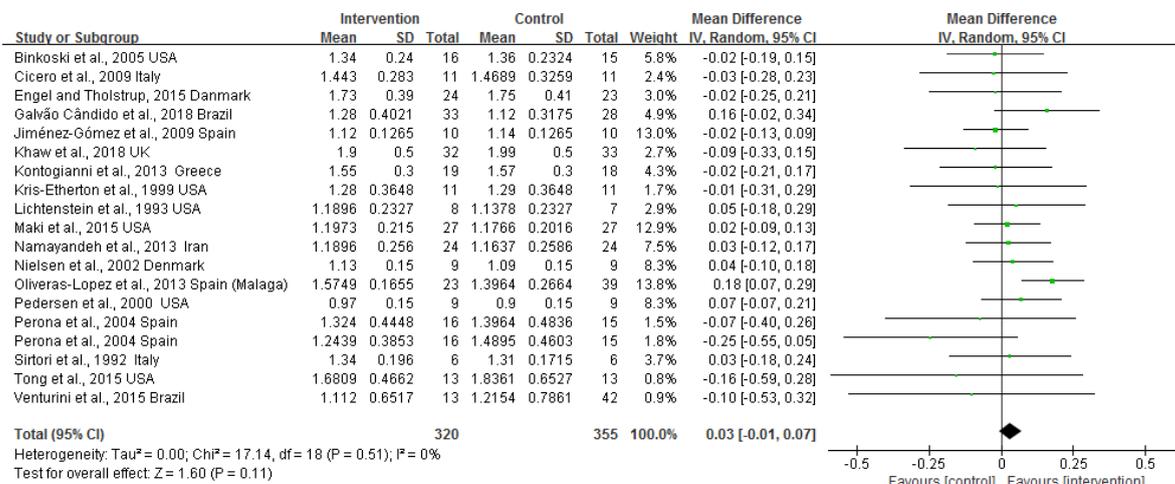


Figure C4. Meta-analysis of studies consuming olive oil on LDL-C (mmol/L).

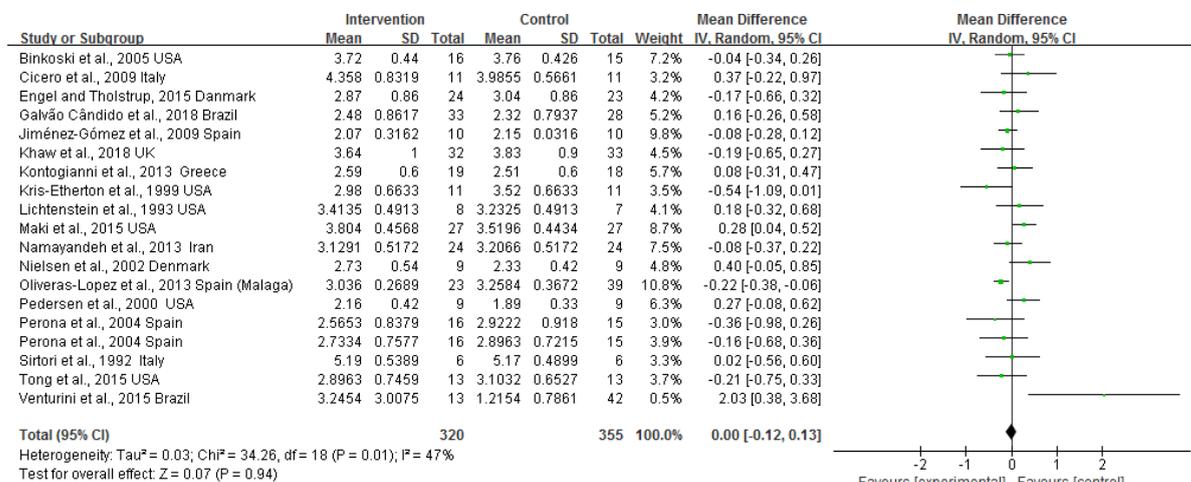


Figure C5. Meta-analysis of studies consuming olive oil on TG (mmol/L).

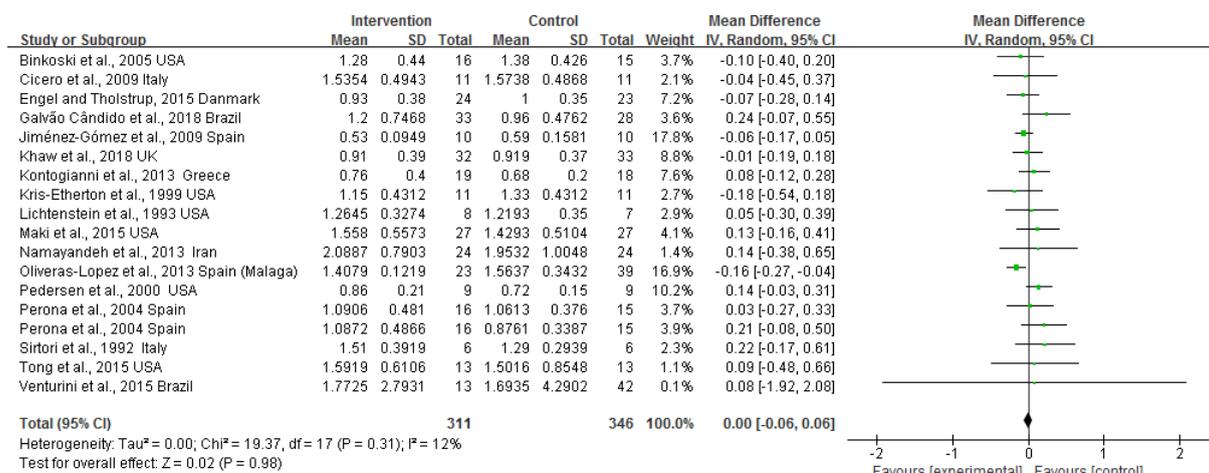


Figure C6. Meta-analysis of studies consuming olive oil on SBP (mmHg).

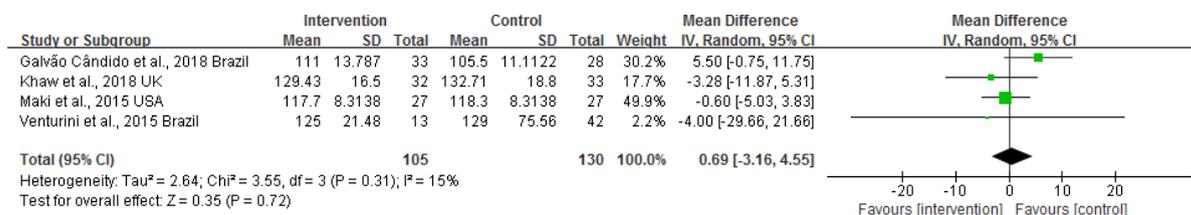


Figure C7. Meta-analysis of studies consuming olive oil on DBP (mmHg).

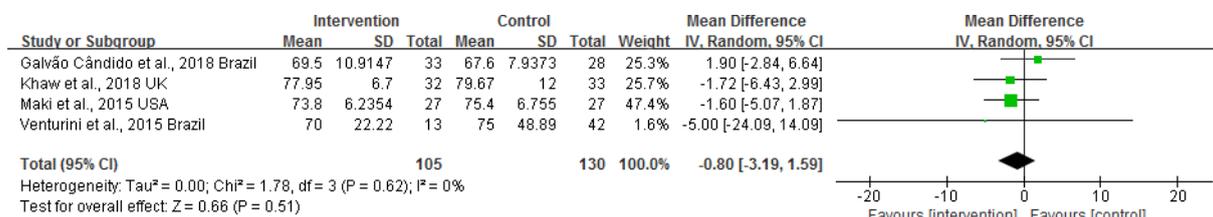


Figure C8. Meta-analysis of studies consuming olive oil on sICAM-1 (ng/mL).

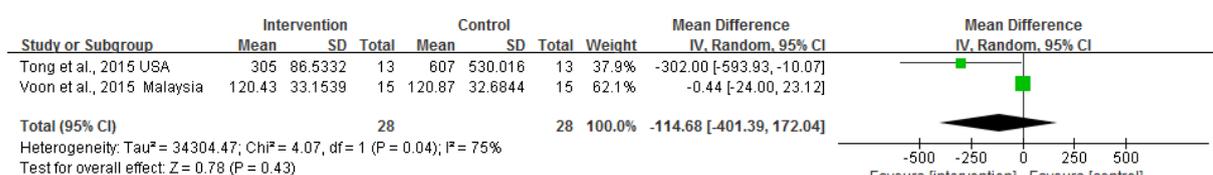


Figure C9. Meta-analysis of studies consuming olive oil on sVCAM-1 (ng/mL).

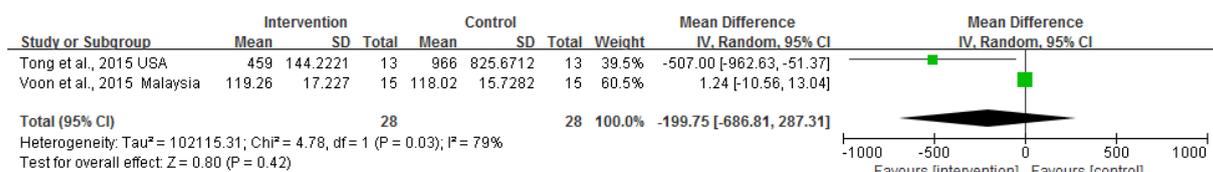


Figure C10. Meta-analysis of studies consuming olive oil on IL-6 (pg/mL).

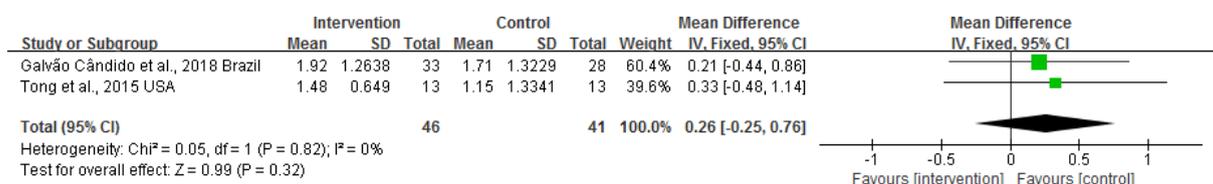


Figure C11. Meta-analysis of studies consuming olive oil on hs-CRP (pg/mL).

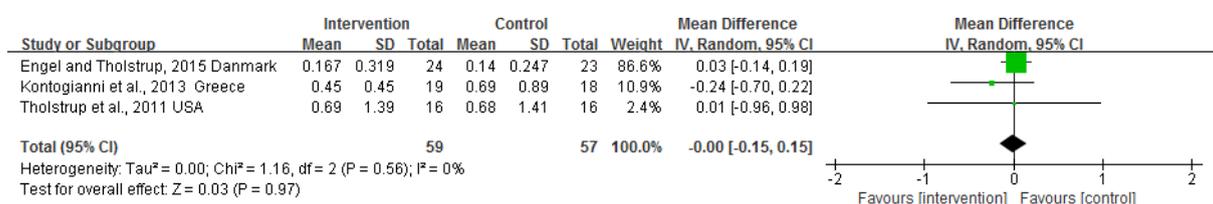


Figure C12. Meta-analysis of studies consuming olive oil on CRP (mg/dL).

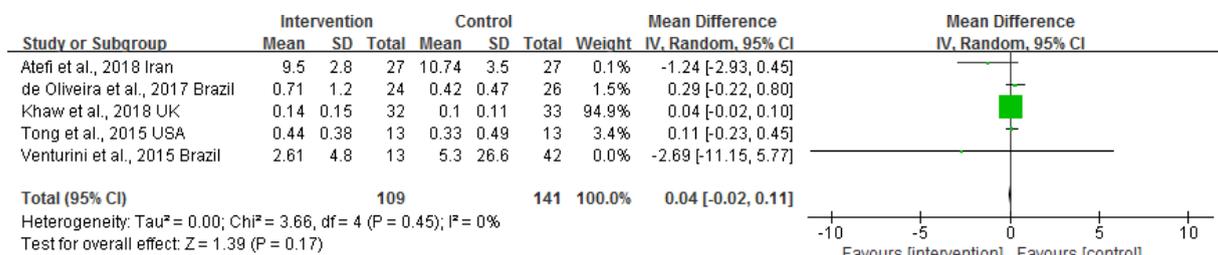


Figure C13. Meta-analysis of studies consuming olive oil on adiponectin (ug/mL).

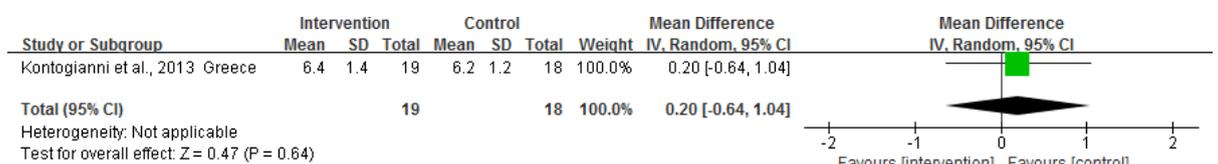


Figure C14. Meta-analysis of studies consuming olive oil on TNF-alpha (pg/mL).

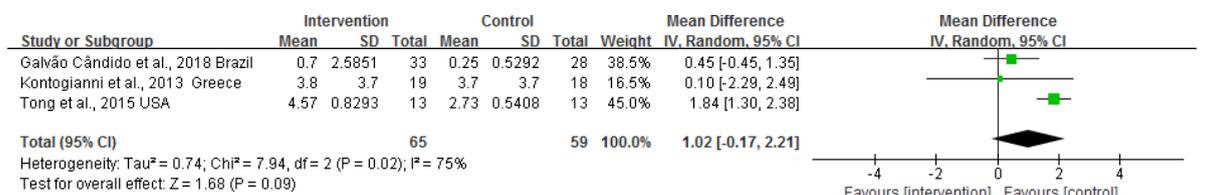


Figure C15. Meta-analysis of studies consuming olive oil on vWF (%).

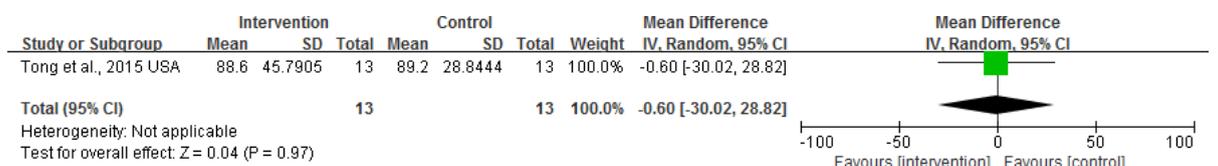


Figure C16. Meta-analysis of studies consuming olive oil on Fibrinogen (g/L).

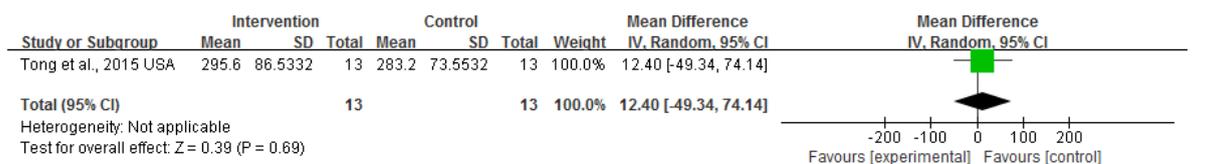


Figure C17. Meta-analysis of studies consuming olive oil on ET-1 (pg/mL).

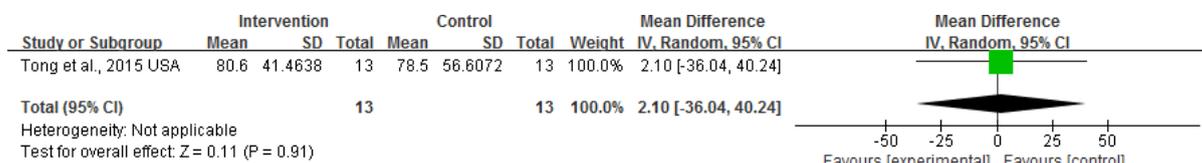


Figure C18. Meta-analysis of studies consuming olive oil on plasma E-Selectin (ng/mL).

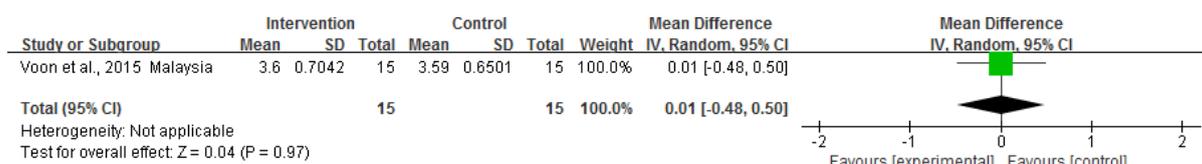


Figure C19. Meta-analysis of studies consuming olive oil on Apo B (mg/dL).

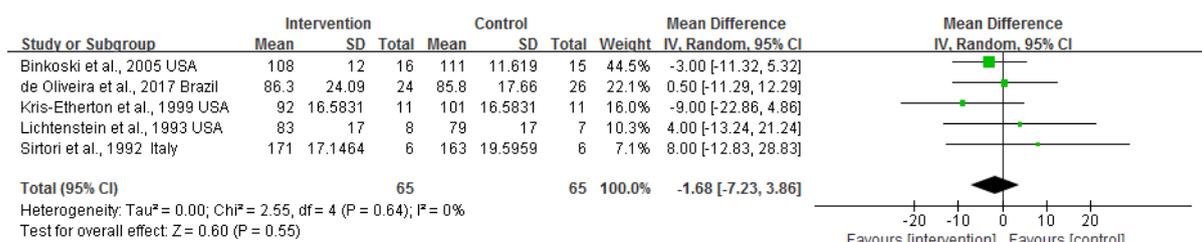


Figure C20. Meta-analysis of studies consuming olive oil on ApoA1 (mg/dL).

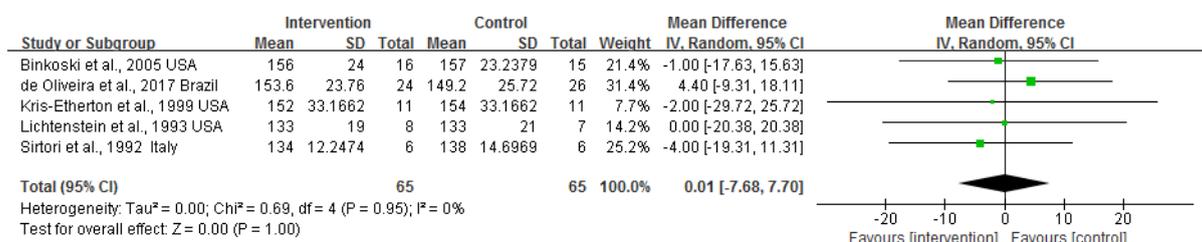


Figure C21. Meta-analysis of studies consuming olive oil on IL-8 (pg/mL).

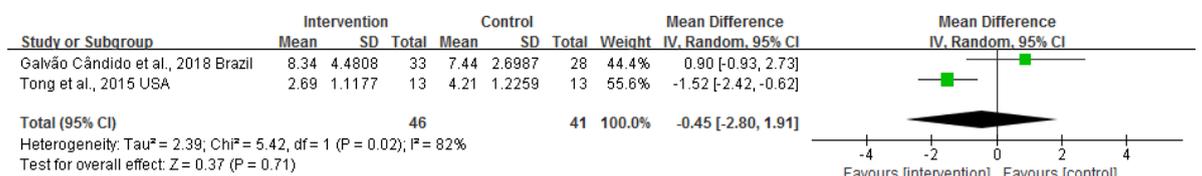
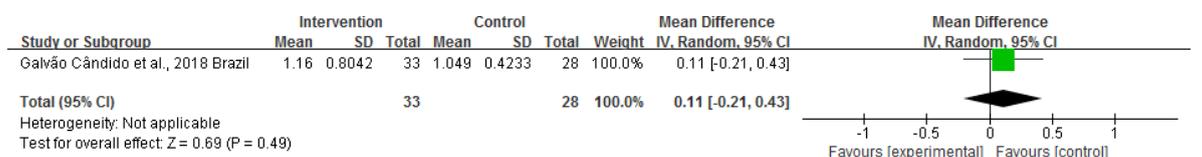


Figure C22. Meta-analysis of studies consuming olive oil on IL-10 (pg/mL).



Appendix D

Supplementary information for Chapter 5

Appendix D1.

Message generated by ClinicalTrials.gov Protocol Registration and Results System

Subject: ClinicalTrials.Protocol Record HLS-JLG-OO study, Effect of Olive Oil Consumption on Cardiovascular Biomarkers, has been reviewed and will be made public on ClinicalTrials.gov.

RECORDS USUALLY APPEAR ON ClinicalTrials.gov WITHIN 2 BUSINESS DAYS of the receipt of this message.

The record has been edited by ClinicalTrials.gov as follows:

Made minor editorial changes

Contact us at: register@clinicaltrials.gov

PRS Team

ClinicalTrials.gov Identifier: **NCT04187638**

Sent: **04 December 2019** 19:35 register@clinicaltrials.gov From: ClinicalTrials.gov Registration

Appendix D2.

Dear Fan Liang,

Submission Ref: **10527**

Following independent peer review of the above proposal*, I am pleased to inform you that **APPROVAL** has been granted on the basis of this proposal and subject to continued compliance with the University policies on ethics, informed consent, and any other policies applicable to your individual research. You should also have current Disclosure & Barring Service (DBS) clearance if your research involves working with children and/or vulnerable adults.

* note: Staff Low Risk applications are auto approved without independent peer review.

The University's Policies and Procedures are [here](#):

All researchers must also notify this office of the following:

1. Any significant changes to the study design, by submitting an 'Ethics Amendment Form'
2. Any incidents which have an adverse effect on participants, researchers or study outcomes, by submitting an 'Ethical incident Form'
3. Any suspension or abandonment of the study.

Please check your approved proposal for any Approval Conditions upon which approval has been made.

Use this link to view the submission: [View Submission](#)

Research Ethics Home: [Research Ethics Home](#)

Appendix D3.

Dear all,

I am studying for a PhD in the final year at Northumbria university in Newcastle. Currently I am looking forward to finding more participants to take part in my study. I will be appreciating if people would like to be interested in my olive oil study.

We are looking for participants either Caucasians or East Asians (healthy male adults ages 18 - 70) to participate in a study investigating the effects of consuming olive oil on cardiovascular markers (4 visits to the clinical room across the intervention).

You will be taking a daily dose of olive oil for two weeks or butter for 2 weeks with 2-week washout period in between. Each visit around 30 minutes. There is a £20 Eldon Square gift voucher to compensate for your time and inconvenience caused. Please contact fan.liang@northumbria.ac.uk for more information.

Visits take place at Northumberland Building (City Campus), if you are interested in my olive oil study trial, please do not hesitate to contact me.

With thanks and kind regards,

Fan

30 Participants needed!

Are you a healthy male adult of Asian or Caucasian origin aged 18-70 years old?

This study has received approval from Northumbria University's Faculty of Health and Life Sciences Ethics Committee

Participants needed for a study examining the effects of consuming olive oil on cardiovascular markers.

The study will involve 4 study visits (around 40 mins each per visit) to our research facilities and taking a daily dose of olive oil for two weeks or butter for 2 weeks with 2-week washout period in between.

Visits take place at Northumberland Building (City Campus)

To compensate for your time, you will receive £20 voucher for taking part in the study

Contact: fan.liang@northumbria.ac.uk for more information.



fan.liang@northumbria.ac.uk

Appendix D5.

Participant Information sheet

TITLE OF PROJECT: Effect of olive oil consumption on cardiovascular biomarkers in Asians and Caucasians: A randomized, crossover, controlled interventional trial

Principal Investigator: Fan Liang

Principal Supervisor: Dr Jose Lara Gallegos

Email: fan.liang@northumbria.ac.uk;

Number of participant payment: £20 voucher will be provided at the end of the study

What is the Purpose of the project?

Olive oil, a central element to the Mediterranean diet, has been associated with beneficial effects on markers of cardiovascular risk.

This study aims to compare the efficacy of consumption of olive oil for two weeks on different cardiovascular risk factors including blood pressure and blood lipids in adult individuals of Asians and Caucasian origin.

Why have I been selected to take part?

You have indicated that you are:

1. Interested in taking part in the study
2. Healthy
3. Aged 18-70 years (inclusive)
4. Either Caucasians or the Orient Asians (such as Chinese, Japanese, Korean, Malaysia Chinese)
5. Willing to consume olive oil or butter in addition to your usual diet during the 6 week supplementation period

What are the exclusion criteria (i.e. are there any reasons why I should not take part)?

You should not take part if you:

- 1. Have been diagnosed and/or are taking medications for hypertension (>140/90mmHg), diabetes, high blood cholesterol and heart problems (e.g. arrhythmia, high-grade stenosis of the carotid artery or carotid sinus syndrome).**
- 2. Are aged below 18 or above 70 years old**
- 3. Have been told to have an allergy to olive oil or olive oil products**
- 4. Have been diagnosed with lactose intolerance**
- 5. Take omega-3 supplements or fish oil and vitamins supplements (regularly during the last six months).**

What will I have to do if I take part in the study?

Key points

1. After signing the consent forms, the researcher will ask you to complete a brief medical and lifestyle questionnaire to assess your general health conditions. Participants will be asked to complete a 3-day food record as well as international physical activity questionnaire to evaluate habitual dietary habits as well as physical status prior to study visits.

2. All study activity will take place at Northumbria University. You will be asked to attend 1 screening visit (about 30 minutes) to confirm you are eligible, and 4 study visits over a 6-week study period to provide blood and urine samples. These visits will be scheduled in the morning and will last for around 40 minutes and will be held in Northumberland building (NB425) at Northumbria University.

3. Before each study visit, you will be required to fast overnight (i.e. please consume your evening meal the day before to the visit before 8:00PM and thereafter only water).

4. You will be asked to consume either olive oil (30ml) for 2 weeks or butter (30g) for 2 weeks, with a 2-week period in between in which you will refrain from consuming any of these. These foods will be incorporated into soup and consumed at lunchtime. These foods will be provided to you.

5. You will be asked to keep a record of your dietary intake for 3 non-consecutive days (i.e. type of foods, preparation and amount of food/drink consumed), in addition you will be required to keep record of your dietary intake during the first 2 weeks of the study and then asked to repeat your food consumption during the next 4 weeks.

6. Independently of the foods to be tested in this study, you will be asked to maintain your usual diet as well as to maintain your usual exercise patterns.

7. Researchers will not be able to provide medical advice to participants. However, participants' results could be provided to your GP if you wish to do so.

General procedure

Pre-screening visit: (approx.. 30minutes)

During this visit, the participants will be asked to read the participant information sheet firstly. Next, participants meeting our inclusion criteria will be asked to sign the consent forms. After you complete the consent forms, the investigator will explain the nature of the research and will ask you to complete pre-screening questionnaire including a 3-day food record, physical activity questionnaire as well as medical and lifestyle questionnaire. At this point, future visit 1 will be scheduled at least three days after this pre-screening visit. Before visit 1 you will be asked to complete a 3-day dietary record.

Study visits 1: (approx. 40 minutes)

You will attend the trial at an agreed time in the morning (for example: 8:00AM), having been fasted from 8:00PM the last evening (12 hours). You will return the completed 3-day dietary record.

Next, we will measure blood pressure, body weight/height without shoes, hand grip strength and body fat. At this session you will also be required to provide a urine sample and fasting venous blood samples. 18ml of fasting venous blood sample (total 72ml for 4 visits) will be taken by a trained researcher. A spot urine sample will be collected into a flask (provided). The whole procedure will be taken within 30 minutes to complete.

Then, you will be given 30ml/day olive oil or 30g/day butter as treatment to consume for two-week duration which you will incorporate into your habitual diet (in combination with

soup during lunch meals). In addition, participants will also be provided with a 3-day dietary record. You will be requested to maintain your usual dietary pattern (i.e. based on the 3- day dietary record you have reported) and usual exercise patterns.

Study visit 2 (approx. 40 minutes)

This appointment will take place 2 weeks after visit 1 (ideally the same day of the week when visit 1 took place). Participants will undergo the same baseline measurements. Fasting urine and venous blood samples will be collected as in the same as visit 1. On arrival, we will check that you have complied with the study. After this visit, you will be asked to avoid olive oil or butter for 2 weeks ('washout' period).

Study visit 3 & 4 (approx. 40 minutes each visit)

Study visit 3 will take place 2 weeks after visit 2. Visit 3 will be identical in procedure as visit 1. Study visit 4 will take place 2 weeks after visit 3. Visit 4 will be identical in procedure as Visit 2. Additionally, you will be debriefed and you will be asked to complete a "Research Participants Experience questionnaire" at the end of Visit 4.

Will my participation involve any physical discomfort?

Venous blood samples will be taken using standard techniques with minor discomfort and will only be carried out by trained phlebotomists (Fan Liang and Jose Lara). However, there might be a slight risk for minor bruising. You will be advised to avoid heavy lifting or strenuous exercise after taking part to minimize bruising.

You will have your blood pressure taken at all appointments using a standard battery operated monitor and cuff. You may find the pressure of the cuff tightening on your arm uncomfortable during this procedure, however this last only a few seconds.

These products to be consumed during this study will be foods commonly eaten and there will be no known side-effects associated with participants' consumption.

Will my participation involve any psychological discomfort or embarrassment?

No. However, we would like you to be aware that in the medical and lifestyle questionnaire include questions related with your health.

5. Will I have to provide any bodily samples (i.e., blood, saliva)?

Yes.

You will be required to provide a fasting venous blood sample and a spot urine sample at each appointment. Blood samples will be taken only after you have given consent to this study for tissue removal, storage and usage. Venous blood sample (18ml for each measurement, total 72ml for 4 visits) will be collected at fasted state. At each visit, you will be also provided with a flask to collect a urine sample in private.

6. Will my taking part in this study be kept confidential and anonymous?

Yes.

You will be allocated a participant code number that will always be used to identify any data that you provide during the study. Only the research team will have access to any identifiable information; paper records will be stored in a locked filing cabinet and electronic information will be stored on a password-protected computer. Any personal details will be kept separate from data and will be treated in accordance with the Data Protection Act.

Who will have access to the information that I provide?

Only the research team (Fan Liang, Jose Lara) will have access to the information that you provide. However, any data that leaves the site will only be identifiable by an identification number and it will not be possible for anybody outside of the investigational site to identify you. Should the results of research be presented or published in any form, then that information will be generalized. Rest assure that your personal information or data will not be identifiable.

How will my information be stored / used in the future?

It is a possible that the results of the study will be published in scientific journals or disseminated in scientific conferences. After the final report has been completed, all study related materials will be archived in accordance with General Data Protection Regulation (GDPR) or the Data Protection Act (DPA) for a minimum of 7 years. At no time you will be identified as having taken part in this study.

Has this investigation received appropriate ethical clearance?

Yes.

This study has received ethical approval from the Faculty of Health and Life Sciences Ethics committee. If you require confirmation of this, please contact the Chair of this Committee, stating the title of the research project and the name of the principal investigator:

Chair of Faculty of Health and Life Sciences Ethics Committee, Northumberland Building, Northumbria University, Newcastle upon Tyne, NE1 8ST

Will I receive any financial rewards / travel expenses for taking part?

Yes. You will receive £20 in vouchers for completing the study.

How can I withdraw from the project?

If you do decide that you do not wish to take any further part of the study, you are free to withdraw from the study at any time, without having to give any reasons and without prejudice. Please inform one of the research team as soon as possible, and they will facilitate your withdrawal and discuss with you how your data will be used in the future. After completion of the study, you can still withdraw your data. However, kindly do so by contacting the research team and give them your participation number within a month of your participation. After this date, it may not be possible to withdraw your individual data as the results may already have been published. As all data are anonymised, your individual data will not be identifiable in any way.

If I require further information who should I contact and how?

If you need further information or wish to withdraw from the study, would like to discuss your participation, or experience any problems as a consequence of taking part in the study you should contact Fan Liang (fan.liang@northumbria.ac.uk) or alternatively, supervisor of the research on jose.lara@northumbria.ac.uk (Office hours).

Appendix D6.

Physical Examination and Intervention Visit Information Form

Physical examination after sitting in an upright position for 5 minutes. A first reading is taken but discarded. If the average reading is out of range, but the third is lower than the second reading, a fourth reading can be taken. This reading alone will then be used as the final measurement. Readings should be taken > 1-minute intervals. If BP is >139-89, subject must be referred to GP.

Project Title: Effect of olive oil consumption on cardiovascular biomarkers in Asians and Caucasians: A randomized, crossover, controlled interventional trial

Principal Investigator: Dr Jose Lara Gallegos

Student Investigator: Fan Liang

Subject ID Number: _____

Date: _____ (Visit number: _____)

Subject initials: _____

Time of arrival: _____

Date of Birth (dd/mm/yyyy): _____

Has subject observed a fast from 20:00 last night? Y/N

Has subject complete 3-day diet recall? Y/N

Anthropometric data:

BP (systolic blood pressure /diastolic blood pressure /heart rate) 1st

reading: _____

2nd reading: _____

3rd reading: _____

4th reading: _____

Height: _____ (m) Weight: _____ (kg) Body Mass Index:
_____ (kg/m²)

Are you a right-handed or left-handed?

Grip strength 1: _____ kg Grip strength 2: _____ kg Grip strength 3:
_____ kg

Body composition:

Body fat: _____ % (normal range: ____ - ____%) Body

fat: _____ kg (normal range: ____ - ____%) Lean:

_____ % (normal range: ____ - ____%)

Lean: _____ kg (normal range: ____ - ____ kg)

Total: _____ kg (normal range: ____ - ____%)

Dry lean weight: _____ kg (normal range: ____ - ____%) Water:

_____ % (normal range: ____ - ____%) Water:

_____ l (normal range: ____ - ____%)

Basal MET.RATE: _____ kcal

BMR: _____ kcal/kg

EST.AVERAGE REQ: _____ kcal

BMI: _____ kg/m² (normal range: ____ - ____%)

Impedance 50KHz: ____Ω

Investigation:

1. Blood sample Y/N

Comment:

2. Urine sample Y/N

Comment:

Researcher sign for confirmation of data completion:

Comment:

Appendix D7.

Study Completion Sheet

Project Title: Effect of olive oil consumption on cardiovascular biomarkers in Asians and Caucasians: A randomized, crossover, controlled interventional trial

Principal Investigator: Dr Jose Lara Gallegos

Student Investigator: Fan Liang

Subject I.D.: _____

Date of Completion/early withdrawal: _____ (dd-mm-yy)

Did the subject complete the study as planned? Yes No

If No, please give primary reason for premature termination / discontinuation

- Adverse event(s) (also specify on the Adverse Event form)
 - Major protocol violation, including non-compliance
 - Subject withdrew consent
 - Lost to follow-up
 - Other please specify:
-

Please provide any relevant information related to the reason for premature discontinuation:

I have reviewed and found all data pertaining to this subject to be complete and accurate:

Principal Investigator's Signature _____

Date: _____(dd-mm-yy)

Research Participants Experience questionnaire

PROJECT TITLE: Effect of olive oil consumption on cardiovascular biomarkers in Asians and Caucasians: A randomized, crossover, controlled interventional trial

RESEARCHER: Fan Liang

PRINCIPAL INVESTIGATOR: Dr Jose Lara Gallegos

We value your opinion on what it was like to be involved in research. We would be grateful if you could complete this anonymous questionnaire about your experience to help us improve in the future.

Subject ID Number: _____(optional)

After taking part in this study, I feel that I will try olive oil more often in the future.

- Strongly Agree
- Agree
- Not Sure
- Disagree
- Strongly Disagree

On a scale of 1-5, with 1 meaning “not at all” and 5 meaning “very much”, how would you rate the researcher (Fan Liang) on the following points? If you did not have any contact with the researcher (emails or attendance of the information sessions) please select NA.

Helpful	1	2	3	4	5

Empathetic	1	2	3	4	5

Personable	1	2	3	4	5

Professional	1	2	3	4	5

Supportive	1	2	3	4	5

Approachable	1	2	3	4	5

I felt valued as a participant in research.

- Strongly Agree
- Agree
- Not Sure
- Disagree
- Strongly Disagree

It is important to me to know the results of a research study.

- Strongly Agree
- Agree
- Not Sure
- Disagree
- Strongly Disagree

I would recommend taking part in a research study to other people.

- Strongly Agree
- Agree
- Not Sure
- Disagree
- Strongly Disagree

Please tell us if there is anything that could have made your overall experience better:

I would be happy to take part in another research study.

1	2	3	4	5

Strongly
Disagree

Please tell us about your reasons for taking part in this study.

Choose all options that apply:

To help others

For my own benefit or care

Health professional recommended it

I am interested in research

Other: _____

Overall, how would you rate your experience of taking part in research?

Very poor

Excellent

1	2	3	4	5	6	7	8	9	10

Thank you for sharing your experiences related to being a research participant.

PARTICIPANT DEBRIEF

Name of Researcher: Fan Liang

Name of Supervisor: Dr Jose Lara Gallegos

Participant Number: _____

Project Title: Effect of olive oil consumption on cardiovascular biomarkers in Asians and Caucasians: A randomized, crossover, controlled interventional trial

1. What was the purpose of the project?

Epidemiological evidence suggests an association between consumption of olive oil and low risk for cardiovascular diseases (CVDs).

How factors such as ethnicity plays a role in this association are not fully investigated. The main aim of this study was to examine the effect of oral olive oil consumption in improving several cardiovascular risk markers in between Caucasians and Asians.

2. How will I find out about the results?

If you require information about the overall results and conclusion of the project simply send an email to the researcher.

3. Have I been deceived in any way during the project?

No. Everything has been stated in the consent form and information form what was required of you and what you had to do to help with this project.

4. If I change my mind and wish to withdraw the information I have provided, how do I do this?

If you wish to withdraw, simply contact Fan Liang (fan.liang@northumbria.ac.uk) with your participant number within a month of your participation. After this date, it may not be possible to withdraw your individual data as the results may already have been published. However, as all data are anonymous, your individual data will not be identifiable in any way.

5. How will the results be disseminated?

The data collected in this study may also be published in scientific journals or presented at scientific conferences. However, rest assured that at no time will you personally be identified.

6. What will happen to the information I have provided?

Your consent forms have been separated from your other data and stored in a locked filing cabinet. The other data will be stored on a password-protected staff PC or in a separate locked filing cabinet until the findings have been published. At no time will you personally be identified as having taken part. We will not provide any information on your own individual performance. After the final report has been completed, all study related materials will be archived in accordance with General Data Protection Regulation (GDPR), for a minimum of 7 years.

If you wish to receive feedback about the findings of this research study then please contact the researcher at **fan.liang@northumbria.ac.uk**

This study and its protocol have received full ethical approval from Faculty of Health and Life Sciences Research Ethics Committee. If you require confirmation of this, or if you have any concerns or worries concerning this research, or if you wish to register a complaint, please contact the Chair of this Committee (Dr Nick Neave: nick.neave@northumbria.ac.uk).

Olive oil consumption self-report sheet

If you take olive oil today, Please place a tick (✓) in the correct box.

Monday	Tuesday	Wednesday	Thursday	Friday	Saturday	Sunday
Take olive oil <input type="checkbox"/>	Take olive oil <input type="checkbox"/>	Take olive oil <input type="checkbox"/>	Take olive oil <input type="checkbox"/>	Take olive oil <input type="checkbox"/>	Take olive oil <input type="checkbox"/>	Take olive oil <input type="checkbox"/>
Take olive oil <input type="checkbox"/>	Take olive oil <input type="checkbox"/>	Take olive oil <input type="checkbox"/>	Take olive oil <input type="checkbox"/>	Take olive oil <input type="checkbox"/>	Take olive oil <input type="checkbox"/>	Take olive oil <input type="checkbox"/>
Take olive oil <input type="checkbox"/>	Take olive oil <input type="checkbox"/>	Take olive oil <input type="checkbox"/>	Take olive oil <input type="checkbox"/>	Take olive oil <input type="checkbox"/>	Take olive oil <input type="checkbox"/>	Take olive oil <input type="checkbox"/>

Take olive oil <input type="checkbox"/> BP measure <input type="checkbox"/> SBP/DBP/HR 1. _____ 2. _____ 3. _____	Take olive oil <input type="checkbox"/> BP measure <input type="checkbox"/> SBP/DBP/HR 1. _____ 2. _____ 3. _____	Take olive oil <input type="checkbox"/> BP measure <input type="checkbox"/> SBP/DBP/HR 1. _____ 2. _____ 3. _____	Take olive oil <input type="checkbox"/> BP measure <input type="checkbox"/> SBP/DBP/HR 1. _____ 2. _____ 3. _____	Take olive oil <input type="checkbox"/> BP measure <input type="checkbox"/> SBP/DBP/HR 1. _____ 2. _____ 3. _____	Take olive oil <input type="checkbox"/> BP measure <input type="checkbox"/> SBP/DBP/HR 1. _____ 2. _____ 3. _____	Take olive oil <input type="checkbox"/> BP measure <input type="checkbox"/> SBP/DBP/HR 1. _____ 2. _____ 3. _____
Take olive oil <input type="checkbox"/> BP measure <input type="checkbox"/> SBP/DBP/HR 1. _____ 2. _____ 3. _____	Take olive oil <input type="checkbox"/> BP measure <input type="checkbox"/> SBP/DBP/HR 1. _____ 2. _____ 3. _____	Take olive oil <input type="checkbox"/> BP measure <input type="checkbox"/> SBP/DBP/HR 1. _____ 2. _____ 3. _____	Take olive oil <input type="checkbox"/> BP measure <input type="checkbox"/> SBP/DBP/HR 1. _____ 2. _____ 3. _____	Take olive oil <input type="checkbox"/> BP measure <input type="checkbox"/> SBP/DBP/HR 1. _____ 2. _____ 3. _____	Take olive oil <input type="checkbox"/> BP measure <input type="checkbox"/> SBP/DBP/HR 1. _____ 2. _____ 3. _____	Take olive oil <input type="checkbox"/> BP measure <input type="checkbox"/> SBP/DBP/HR 1. _____ 2. _____ 3. _____

Butter consumption self-report sheet

If you take olive oil today, Please place a tick (✓) in the correct box.

Monday	Tuesday	Wednesday	Thursday	Friday	Saturday	Sunday
Take butter <input type="checkbox"/>	Take butter <input type="checkbox"/>	Take butter <input type="checkbox"/>	Take butter <input type="checkbox"/>	Take butter <input type="checkbox"/>	Take butter <input type="checkbox"/>	Take butter <input type="checkbox"/>
Take butter <input type="checkbox"/>	Take butter <input type="checkbox"/>	Take butter <input type="checkbox"/>	Take butter <input type="checkbox"/>	Take butter <input type="checkbox"/>	Take butter <input type="checkbox"/>	Take butter <input type="checkbox"/>
Take butter <input type="checkbox"/>	Take butter <input type="checkbox"/>	Take butter <input type="checkbox"/>	Take butter <input type="checkbox"/>	Take butter <input type="checkbox"/>	Take butter <input type="checkbox"/>	Take butter <input type="checkbox"/>
Take butter <input type="checkbox"/> BP measure <input type="checkbox"/> SBP/DBP/HR 1. _____ 2. _____ 3. _____	Take butter <input type="checkbox"/> BP measure <input type="checkbox"/> SBP/DBP/HR 1. _____ 2. _____ 3. _____	Take butter <input type="checkbox"/> BP measure <input type="checkbox"/> SBP/DBP/HR 1. _____ 2. _____ 3. _____	Take butter <input type="checkbox"/> BP measure <input type="checkbox"/> SBP/DBP/HR 1. _____ 2. _____ 3. _____	Take butter <input type="checkbox"/> BP measure <input type="checkbox"/> SBP/DBP/HR 1. _____ 2. _____ 3. _____	Take butter <input type="checkbox"/> BP measure <input type="checkbox"/> SBP/DBP/HR 1. _____ 2. _____ 3. _____	Take butter <input type="checkbox"/> BP measure <input type="checkbox"/> SBP/DBP/HR 1. _____ 2. _____ 3. _____

Take butter <input type="checkbox"/> BP measure <input type="checkbox"/> SBP/DBP/HR 1. _____ 2. _____ 3. _____ _____	Take butter <input type="checkbox"/> BP measure <input type="checkbox"/> SBP/DBP/HR 1. _____ 2. _____ 3. _____	Take butter <input type="checkbox"/> BP measure <input type="checkbox"/> SBP/DBP/HR 1. _____ 2. _____ 3. _____	Take butter <input type="checkbox"/> BP measure <input type="checkbox"/> SBP/DBP/HR 1. _____ 2. _____ 3. _____	Take butter <input type="checkbox"/> BP measure <input type="checkbox"/> SBP/DBP/HR 1. _____ 2. _____ 3. _____	Take butter <input type="checkbox"/> BP measure <input type="checkbox"/> SBP/DBP/HR 1. _____ 2. _____ 3. _____	Take butter <input type="checkbox"/> BP measure <input type="checkbox"/> SBP/DBP/HR 1. _____ 2. _____ 3. _____
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Adverse Event Report

Project Title: Effect of olive oil consumption on cardiovascular biomarkers in Asians and Caucasians: A randomized, crossover, controlled interventional trial		
Name of Researcher: Fan Liang	Participant ID Number: _____ - _____	Visit Date: _____ (mm/dd/yy)
Name of Supervisor: Dr Jose Lara Gallegos	_____	

Has the subject had any Adverse Events during this study? Yes No (If yes, please list all Adverse Events below)

					Event Analysis			
Adverse Event Description	Start Date (mm/dd/yy)	Severity	Action Taken	Stop Date (mm/dd/yy) OR Check continuing <input type="checkbox"/> If	Relationship to Study Intervention possible?	Expected?	Treatment of Adverse Event?	Final Outcome of Event
1.				<input type="checkbox"/>				
2.				<input type="checkbox"/>				

3.					<input type="checkbox"/>				
4.					<input type="checkbox"/>				
5.					<input type="checkbox"/>				
6.					<input type="checkbox"/>				
7.					<input type="checkbox"/>				
8.					<input type="checkbox"/>				
9.					<input type="checkbox"/>				
10.					<input type="checkbox"/>				
11.					<input type="checkbox"/>				

12.					<input type="checkbox"/>				
13.					<input type="checkbox"/>				
14.					<input type="checkbox"/>				
15.					<input type="checkbox"/>				
16.					<input type="checkbox"/>				

Severity: 1 = Mild, 2 = Moderate, 3 = Severe;

Action Taken: 1 = None, 2 = Discontinued permanently, 3 = Interrupted temporarily, 4 = Reduced Dose,
5 = Increased Dose, 6 = Delayed Dose

Final Outcome of AE: 1 = Resolved, No Sequelae, 2 = AE still present- no treatment, 3 = AE still present-being treated, 4 = Residual effects present-not treated,
5 = Residual effects present- treated, 6 = Death, 7 = Unknown

Relationship to Study Intervention: 1 = Definitely related, 2 = Possibly related, 3 = Not related

Expected: 1 = Yes 2 = No

Appendix Table D8. CONSORT 2010 checklist of information to include when reporting a randomised trial.

Section/Topic	Item No	Checklist item	Reported on page No
Title and abstract			
	1a	Identification as a randomized trial in the title	156
	1b	Structured summary of trial design, methods, results, and conclusions (for specific guidance see CONSORT for abstracts)	N/A
Introduction			
Background and objectives	2a	Scientific background and explanation of rationale	157 - 158
	2b	Specific objectives or hypotheses	158
Methods			
Trial design	3a	Description of trial design (such as parallel, factorial) including allocation ratio	161 - 162
Participants	4a	Eligibility criteria for participants	162 - 163
	4b	Settings and locations where the data were collected	163
Interventions	5	The interventions for each group with sufficient details to allow replication, including how and when they were actually administered	163 - 164
Outcomes	6a	Completely defined pre-specified primary and secondary outcome measures, including how and when they were assessed	167
	6b	Any changes to trial outcomes after the trial commenced, with reasons	N/A
Sample size	7a	How sample size was determined	172
	7b	When applicable, explanation of any interim analyses and stopping guidelines	172

Randomization			
Sequence generation	8a	Method used to generate the random allocation sequence	164 - 165
	8b	Type of randomization; details of any restriction (such as blocking and block size)	164 - 165
Allocation concealment mechanism	9	Mechanism used to implement the random allocation sequence (such as sequentially numbered containers), describing any steps taken to conceal the sequence until interventions were assigned	164- 165
Implementation	10	Who generated the random allocation sequence, who enrolled participants, and who assigned participants to interventions	164
Blinding	11a	If done, who was blinded after assignment to interventions (for example, participants, care providers, those assessing outcomes) and how	164
	11b	If relevant, description of the similarity of interventions	N/A
Statistical methods	12a	Statistical methods used to compare groups for primary and secondary outcomes	167- 173
	12b	Methods for additional analyses, such as subgroup analyses and adjusted analyses	173
Results			
Participant flow (a diagram is strongly recommended)	13a	For each group, the numbers of participants who were randomly assigned, received intended treatment, and were analyzed for the primary outcome	174 - 189
	13b	For each group, losses and exclusions after randomization, together with reasons	174 - 189
Recruitment	14a	Dates defining the periods of recruitment and follow-up	163 - 164
	14b	Why the trial ended or was stopped	N/A
Baseline data	15	A table showing baseline demographic and clinical characteristics for each group	175 - 176

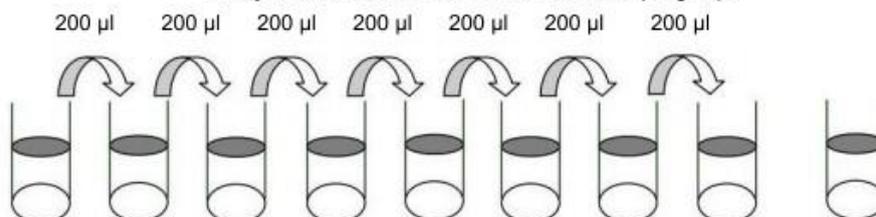
Numbers analyzed	16	For each group, number of participants (denominator) included in each analysis and whether the analysis was by original assigned groups	178
Outcomes and estimation	17a	For each primary and secondary outcome, results for each group, and the estimated effect size and its precision (such as 95% confidence interval)	180- 189
	17b	For binary outcomes, presentation of both absolute and relative effect sizes is recommended	180- 189
Ancillary analyses	18	Results of any other analyses performed, including subgroup analyses and adjusted analyses, distinguishing pre-specified from exploratory	180- 189
Harms	19	All-important harms or unintended effects in each group (for specific guidance see CONSORT for harms)	N/A
Discussion			
Limitations	20	Trial limitations, addressing sources of potential bias, imprecision, and, if relevant, multiplicity of analyses	197- 199
Generalizability	21	Generalizability (external validity, applicability) of the trial findings	191 -192
Interpretation	22	Interpretation consistent with results, balancing benefits and harms, and considering other relevant evidence	193 -199
Other information			
Registration	23	Registration number and name of trial registry	161
Protocol	24	Where the full trial protocol can be accessed, if available	N/A
Funding	25	Sources of funding and other support (such as supply of drugs), role of funders	N/A

Appendix D9. Manufacturer's instructions for Human P-selectin ELISA kit.

Certificate of Analysis / Protocol	3050 Spruce Street, Saint Louis, MO 63103 USA Tel: (800) 521-8956 (314) 771-5765 Fax: (800) 325-5052 (314) 771-5757
Product Name	Human P-Selectin ELISA Kit for serum, plasma, and cell culture supernatants
Product Number	RAB0426
Lot Number	0509F0217
Storage	Store the kit at -20°C. It remains active for up to 1 year. Avoid repeated freeze-thaw cycles. The reconstituted standard should be stored at -20°C or -70°C (-70°C is recommended). Opened microplate strips or reagents may be stored for up to 1 month at 2-8°C. Return unused wells to the pouch containing desiccant pack and reseal along entire edge.
Components	<ol style="list-style-type: none">1. Human P-Selectin Antibody-coated ELISA Plate (Item A) - RAB0426A-EA: 96 wells (12 strips x 8 wells) coated with anti-Human P-Selectin.2. 20x Wash Buffer (Item B) - RABWASH43. Lyophilized Human P-Selectin Protein Standard (Item C) - RAB0426C-1VL4. Biotinylated Human P-Selectin Detection Antibody (Item F) - RAB0426D-1VL5. HRP-Streptavidin (Item G) - RABHRP56. ELISA Colorimetric TMB Reagent (HRP Substrate, Item H) - RABTMB37. ELISA Stop Solution (Item I) - RABSTOP38. ELISA 1x Assay/Sample Diluent Buffer A (Item D1) - RABELADA-30ML9. ELISA 5x Assay/Sample Diluent Buffer B (Item E1) - RABELADB-15ML
Assay/Sample Diluent Buffer dilution (Preparation, Step 2)	Assay/Sample Diluent Buffer B (Item E1) should be diluted 5-fold with deionized or distilled water before use.
Sample Dilution (Preparation, Step 3)	Assay/Sample Diluent Buffer A (Item D1) should be used for dilution of serum and plasma samples. 1x Assay/Sample Diluent Buffer B (Item E1) should be used for dilution of cell culture supernatant samples. The suggested dilution for normal serum/plasma is 50 - 500 fold. * Please note that the levels of P-Selectin may vary between different samples. Optimal dilution factors for each sample must be determined by the investigator.

**Preparation of Standard
(Preparation, Step 4)**

Briefly spin a vial of Item C. Add 400 µl Assay Diluent A (for serum/plasma samples) or 1X Assay Diluent B (for cell culture medium, Assay Diluent B should be diluted 5-fold with deionized or distilled water) into Item C vial to prepare a 100 ng/ml standard. Dissolve the powder thoroughly by a gentle mix. Add 200 µl P-Selectin standard from the vial of Item C, into a tube with 466.7 µl Assay Diluent A or 1X Assay Diluent B to prepare a 30 ng/ml stock standard solution. Pipette 400 µl Assay Diluent A or 1X Assay Diluent B into each tube. Use the stock standard solution to produce a dilution series (Figure 1). Mix each tube thoroughly before the next transfer. Assay Diluent A or 1X Assay Diluent B serves as the zero standard (0 ng/ml).



		Std1	Std2	Std3	Std4	Std5	Std6	Std7		Zero Standard
Diluent volume	Item C+ 400 µl	466.7 µl	400 µl	400 µl	400 µl	400 µl	400 µl	400 µl		400 µl
Conc.	100 ng/ml	30 ng/ml	10 ng/ml	3.333 ng/ml	1.111 ng/ml	0.37 ng/ml	0.123 ng/ml	0.041 ng/ml		0 ng/ml

**Preparation of Biotinylated
Detection Antibody
(Preparation, Step 6)**

Briefly spin the Detection Antibody vial (Item F) before use. Add 100 µl of 1x Diluent Buffer B (Item E1) into the vial to prepare a detection antibody concentrate. Pipette up and down to mix gently (the concentrate can be stored at 4°C for 5 days). The detection antibody concentrate should be diluted 80-fold with 1x Diluent Buffer B (Item E1) and used in Procedure, step 4.

**Dilution of HRP-Streptavidin
Concentrate
(Preparation, Step 7)**

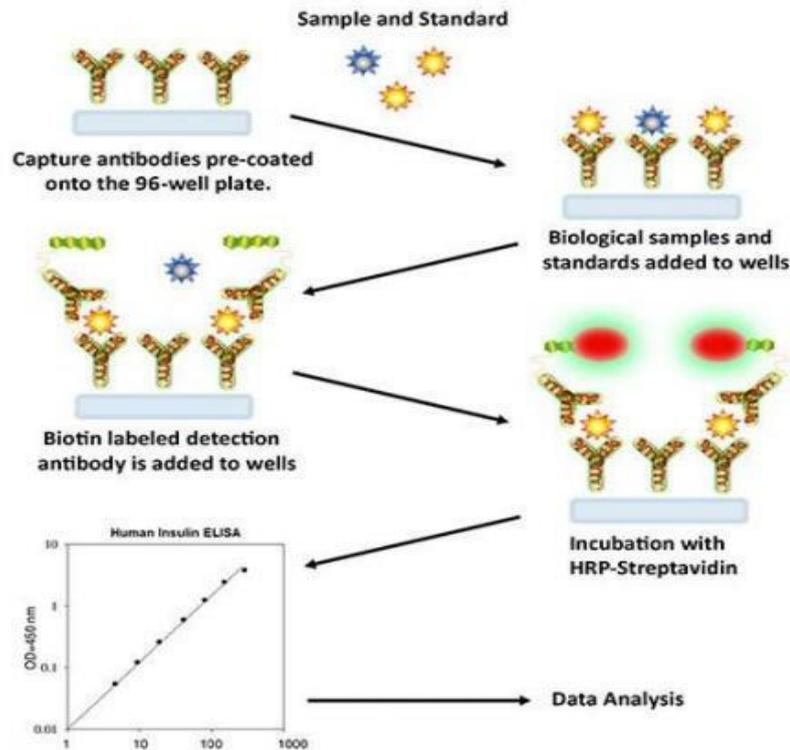
Briefly spin the HRP-Streptavidin concentrate vial (Item G) and pipette up and down to mix gently before use, as precipitates may form during storage. HRP-Streptavidin concentrate should be diluted 800-fold with 1x Diluent Buffer B (Item E1).

For example: Briefly spin the vial (Item G) and pipette up and down to mix gently. Add 20 µl of HRP-Streptavidin concentrate into a tube with 16 ml 1X Assay Diluent B to prepare a 800-fold diluted HRP- Streptavidin solution (don't store the diluted solution for next day use). Mix well.

Sandwich Assay Procedure

1. Bring all reagents and samples to room temperature (18 - 25°C) before use. It is recommended that all standards and samples be run at least in duplicate.
2. Add 100 µl of each standard and sample into appropriate wells. Cover wells and incubate for 2.5 hours at room temperature or overnight at 4°C with gentle shaking.
3. Discard the solution and wash 4 times with 1X Wash Solution. Wash by filling each well with Wash Buffer (300 µl) using a multi-channel Pipette or autowasher. Complete removal of liquid at each step is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against clean paper towels.
4. Add 100 µl of 1x prepared Detection Antibody to each well. Cover wells and incubate for 1 hour at room temperature with gentle shaking.
5. Discard the solution. Repeat the wash procedure as in step 3.
6. Add 100 µl of prepared Streptavidin solution to each well. Cover wells and incubate for 45 minutes at room temperature with gentle shaking.
7. Discard the solution. Repeat the wash as in step 3.
8. Add 100 µl of TMB One-Step Substrate Reagent (Item H) to each well. Cover wells and incubate for 30 minutes at room temperature in the dark with gentle shaking.
9. Add 50 µl of Stop Solution (Item I) to each well. Read absorbance at 450 nm immediately.

Fig 2: Example of the Sandwich ELISA process



Sigma-Aldrich Human P-selectin ELISA kit Procedure

Bring all reagents and samples to room temperature (18–25 °C) before use. Samples and ELISA kit need to take out before use 8 hours.

Sample diluent preparation:

30 – 4= 26 ml

26 / 88 = 0.28 ml Diluent Buffer A (pipette firstly)

$x/(0.28+x) = 1/57$ $x = 0.005$ ml = 5ul sample

57 fold – 5ul to 280 ul of diluent buffer A; in total 285 ul.

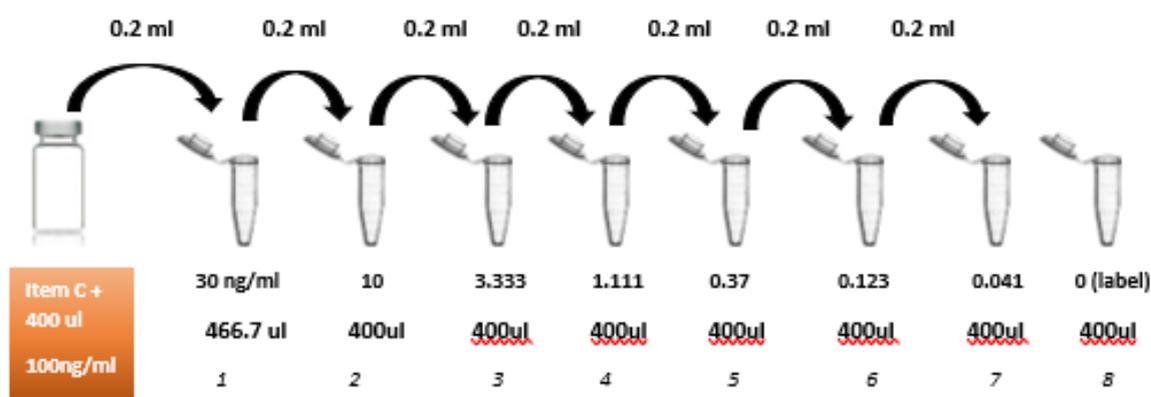
$X / (0.24ml+x) = 1/61$ $x=4$ **ul:** 4 ul of sample, 0.24ml of sample, 61fold

Preparation of Standard:

1. Spin vial of Protein Standard (Item C):
2. Pipette 400 ul Assay Diluent A into **Item C vial** $466.7 + 400*9=3600ul = 4.0667$ ml
3. Gentle Mix. Add 200 ul from **vial Item C** into a tube with 466.7 ul Diluent A (to prepare a 30ng/ml stock standard solution)
4. Pipette 400 ul Assay Diluent A into each 7 labelled **tube**

	Standard:	Add:	Into:
	100 ng/ml (standard solution)	Describe above	
1	30 ng/ml	0.2 ml of the 100 ng/ml std.	0.4667 ml of the diluent A
2	10 ng/ml	0.2 ml of the 30	0.4 ml of the diluent A
3	3.333	0.2 ml of the 10	0.4 ml of the diluent A
4	1.111	0.2 ml of the 3.333	0.4 ml of the diluent A
5	0.37	0.2 ml of the 1.111	0.4 ml of the diluent A
6	0.123	0.2 ml of the 0.37	0.4 ml of the diluent A
7	0.041	0.2 ml of the 0.123	0.4 ml of the diluent A
8	0		0.4 ml of the diluent A

Figure 1: *Assay Diluent A serves as the zero standard (0 pg/ml)



Add **100 µl** of each standard, sample or control into appropriate wells. Cover wells and incubate for **2.5 hours** at room temperature or overnight at 4 C **[with gentle shaking]**.

Assay/sample Diluent buffer B dilution:

1 volume of sample diluent buffer B (**Item E1**) with 4 volume of deionized/distilled water before use.

e.g. 15ml of **Item E1** ~ 60ml of deionized water = 75 ml in total of **Item E1**

Label as Working Diluent Buffer B

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E319, put in TECAN SPARK 10M to gently shake.

If the Wash Buffer (20x) (**Item B**) contains visible crystals, warm to room temperature and mix gently until any precipitated salts have dissolved. Dilute 25 ml of Wash Buffer concentrate into deionized or distilled water to yield **500 ml of 1x Wash Buffer**.

Add 25 ml of the Wash Buffer concentrate (20x) into **475 ml** of deionized water.

Label as Working Wash Buffer.

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Store both concentrate and the working wash buffer in the refrigerator, the dilute buffer should be used within 14 days.

Preparation of Biotinylated Detection Antibody (Item F**):**

1. Spin **Item F** vial.

2. Add 100ul Diluent B **Item E1** into the **Item F** vial.

3. Pipette up and down to mix gently (the concentrate can be stored at 4 for 5 days).

$1/80 = 100 / (x+100)$ $x = 7900$ ul **Item E1** **15800 ul**

Label as Working Detection Antibody.

Dilution of HRP-Streptavidin Concentrate (Item G**) (Within 15 minutes of usage)**

Spin **Item G** – pipette up and down to mix gently before use, as precipitates may form during storage.

Item G should be diluted **800 fold** with 1* Diluent Buffer B (**Item E1**)

Pipette 20 ul of **Item G** into a tube with 16 ml of 1* **Item E1**, mix well.

Label as Streptavidin-HRP working solution.

Thoroughly aspirate or decant the solution and wash 3 times with Working Wash Buffer (300ul).

Wash by filling each well with Wash Buffer (300 µl) using an auto-washer or a squirt bottle. Let soak for 15-30 seconds, then aspirate the liquid 吸液 then repeat.

Squirt bottle. If squirt bottle is used, flood the plate with wash buffer, completely filling all wells. After the washing procedure, the plate is inverted, blot it against the clean paper towels 用干净的纸巾吸干 or and tapped dry on absorbent tissue.

Or use the multi-pipette and tray

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Pipette 100 µl of 1x prepared Detection Antibody = Biotinylated anti-(Biotin Conjugate) to each well. Cover wells and incubate for 1 hour at room temperature with gentle shaking.

Thoroughly aspirate or decant solution from wells and discard the liquid.

Add 100 µl of prepared Streptavidin Working solution to each well. Cover wells and incubate for 45 minutes at room temperature with gentle shaking.

Thoroughly aspirate or decant solution from wells and discard the liquid.

Add 100 µl of TMB One-Step Substrate Reagent = Stabilized Chromogen = HRP Substrate (Item H) to each well. Cover wells and incubate for 30 minutes at room temperature in the dark with gentle shaking. The liquid in the wells will begin to turn blue.

Add 50 µl of Stop Solution (Item I) to each well. Read the absorbance of each well at 450 nm immediately (within 2 hours) having blanked the plate reader against a chromogen blank composed of substrate and stop solution.

Determine the optical density of each well within 30 minutes, using a microplate reader set to 450 nm having blanked the plate reader against a chromogen blank composed of 100ul each of Stabilized Chromogen and stop solution.

The minimum detectable dose of Human CRP was determined to be 30 µg/ml.

Results

Calculations

Calculate the mean absorbance for each set of duplicate standards, controls, and samples, and subtract the average zero standard optical density. Plot the standard curve using SigmaPlot software, with standard concentration on the x-axis and absorbance on the y-axis. Draw the best-fit curve through the standard points.

Yellow: visit 2; Green: visit 4; Grapefruit: visit 1; Gray: visit 3

Data Templates

	1	2	3	4	5	6	7	8	9	10	11	12
A	30	P1V2	P9 V2	P17 V2	P25 V2	P1V4	P9 V4	P17 V4	P25 V4	P1V1	P21V1	P5V3
B	10	P2V2	P10 V2	P18 V2	P26 V2	P2V4	P10 V4	P18 V4	P26 V4	P2V1	P22V1	P6V3
C	3.333	P3V2	P11 V2	P19 V2	P27 V2	P3V4	P11 V4	P19 V4	P27 V4	P3V1	P23V1	P19V3
D	1.111	P4V2	P12 V2	P20 V2	P28 V2	P4V4	P12 V4	P20 V4	P28 V4	P4V1	P24V1	P20V3
E	0.37	P5V2	P13 V2	P21 V2	P29 V2	P5V4	P13 V4	P21 V4	P29 V4	P5V1	P1V3	P21V3
F	0.123	P6V2	P14 V2	P22 V2	P30 V2	P6V4	P14 V4	P22 V4	P30 V4	P6V1	P2V3	P22V3
G	0.041	P7V2	P15 V2	P23 V2	P31 V2	P7V4	P15 V4	P23 V4	P31 V4	P19V1	P3V3	P23V3
H	0	P8V2	P16 V2	P24 V2	P32 V2	P8V4	P16 V4	P24 V4	P32 V4	P20V1	P4V3	P24V3

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fan.liang

I will suggest that you include 12 samples from visit 1 and 12 samples from visit 3. These samples should belong to the same participants (for example participant 1, 2,3,4, etc for visit one, and also participants 1,2,3,4, etc for visit 3).

So, we will have 32 samples for visit 2, 32 samples for visit 4, 12 samples for visit 1, 12 samples for visit 3, and 8 standard curve. Total of 96, which is the number of wells we have in a microplate.

#8-well strips

CB: Chromogen blank 色原空白

	1	2	3	4	5	6	7	8	9	10	11	12
A	30	MM2	LuP2	STC2	ZJN2	MM4	LuP 4	STC 4	ZJN 4	MM1	LWD1	AD3
B	10	FG2	NN2	AH2	JR2	FG 4	NN 4	AH 4	JR 4	FG1	WL1	RD3
C	3.333	YY2	MC2	DDN2	JC2	YY 4	MC 4	DDN 4	JC 4	YY1	AW1	DDN3
D	1.111	JH2	JHE2	KTC2	RHB2	JH 4	JHE 4	KTC 4	RHB 4	JH1	HMC1	KTC3
E	0.37	AD2	TS2	LWD2	YZ2	AD 4	TS 4	LWD 4	YZ 4	AD1	MM3	LWD3
F	0.123	RD2	IJ2	WL2	BRR2	RD 4	IJ 4	WL 4	BRR 4	RD1	FG3	WL3
G	0.041	JBT2	SC2	AW2	XL2	JBT 4	SC 4	AW 4	XL 4	DDN1	YY3	AW3
H	0	SS2	JJ2	HMC2	RH2	SS 4	JJ 4	HMC 4	RH 4	KTC1	JH3	HMC3

<>	1	2	3	4	5	6	7	8	9	10	11	12
A	1.4012	0.7767	0.8628	0.5616	1.2984	0.7564	0.6573	0.5105	0.5189	0.6407	0.7387	0.7158
B	1.3143	0.6507	0.7244	0.701	1.043	0.6291	0.6636	0.5727	0.5393	0.5193	0.5454	0.4589
C	1.1957	0.7104	0.6682	0.8416	0.7313	0.5723	0.6062	0.5141	0.4677	0.7102	0.5494	0.5142
D	1.0065	0.5991	0.7282	0.6451	0.9049	0.5276	0.5752	0.5404	0.5265	0.6887	0.488	0.4643
E	0.7078	0.6355	0.6174	0.574	0.6219	0.6951	0.5042	0.4526	0.4355	0.4732	0.5116	0.3724
F	0.5437	0.6625	0.7366	0.7496	0.6668	0.5286	0.5306	0.3679	0.3769	0.3991	0.4247	0.282
G	0.477	0.5603	0.7239	0.4942	0.6506	0.541	0.4638	0.3576	0.3988	0.5634	0.4924	0.2541
H	0.4643	0.5731	0.5084	0.5773	0.7267	0.5131	0.4683	0.4735	0.5101	0.7073	0.5951	0.4023

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<>	1	2	3	4	5	6	7	8	9	10	11	12
A	0.9369	0.5267	0.6128	0.3116	1.0484	0.5064	0.4073	0.2605	0.2689	0.3907	0.4887	0.4658
B	0.5314	0.4007	0.4744	0.451	0.793	0.3791	0.4136	0.3227	0.2893	0.2693	0.2954	0.2089
C	0.2435	0.4604	0.4182	0.5916	0.4813	0.3223	0.3562	0.2641	0.2177	0.4602	0.2994	0.2642
D	0.1025	0.3491	0.4782	0.3951	0.6549	0.2776	0.3252	0.2904	0.2765	0.4387	0.238	0.2143
E	0.0794	0.3855	0.3674	0.324	0.3719	0.4451	0.2542	0.2026	0.1855	0.2232	0.2616	0.1224
F	0.0287	0.4125	0.4866	0.4996	0.4168	0.2786	0.2806	0.1179	0.1269	0.1491	0.1747	0.032
G	0.0127	0.3103	0.4739	0.2442	0.4006	0.291	0.2138	0.1076	0.1488	0.3134	0.2424	0.0041
H	0	0.3231	0.2584	0.3273	0.4767	0.2631	0.2183	0.2235	0.2601	0.4573	0.3451	0.1523

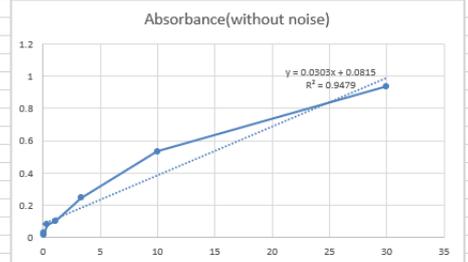
x= (y-0.0815)/ 0.0303

<>	1	2	3	4	5	6	7	8	9	10	11	12
A	30	14.69307	17.53465	7.594059	31.91089	14.0231	10.75248	5.907591	6.184818	10.20462	13.43894	12.68317
B	10	10.53465	12.967	12.19472	23.48185	9.821782	10.9604	7.960396	6.858086	6.19802	7.059406	4.20462
C	3.333	12.50495	11.11221	16.83498	13.19472	7.947195	9.066007	6.026403	4.49505	12.49835	7.191419	6.029703
D	1.111	8.831683	13.09241	10.34983	18.92409	6.471947	8.042904	6.894389	6.435644	11.78878	5.165017	4.382838
E	0.37	10.033	9.435644	8.0033	9.584158	12	5.69967	3.9967	3.432343	4.676568	5.943894	1.349835
F	0.123	10.92409	13.36964	13.79868	11.06601	6.50495	6.570957	1.20132	1.49835	2.231023	3.075908	1.633663
G	0.041	7.551155	12.9505	5.369637	10.53135	6.914191	4.366337	0.861386	2.221122	7.653465	5.310231	2.554455
H	0	7.973597	5.838284	8.112211	13.0429	5.993399	4.514851	4.686469	5.894389	12.40264	8.69967	2.336634

<>	1	2	3	4	5	6	7	8	9	10	11	12
A	30	896.28	1069.61	463.24	1946.56	855.41	655.90	360.36	377.27	622.48	819.78	773.67
B	10	642.61	790.99	743.88	1432.39	599.13	668.58	485.58	418.34	378.08	430.62	256.48
C	3.333	762.80	677.84	1026.93	804.88	484.78	553.03	367.61	274.20	762.40	438.68	367.81
D	1.111	538.73	798.64	631.34	1154.37	394.79	490.62	420.56	392.57	719.12	315.07	267.35
E	0.37	612.01	575.57	488.20	584.63	732.00	347.68	243.80	209.37	285.27	362.58	82.34
F	0.123	666.37	815.55	841.72	675.03	396.80	400.83	73.28	91.40	136.09	187.63	99.65
G	0.041	460.62	789.98	327.55	642.41	421.77	266.35	52.54	135.49	466.86	323.92	155.82
H	0	486.39	356.14	494.84	795.62	365.60	275.41	285.87	359.56	756.56	530.68	142.53

Concentration (ng/ml)	Absorbance(without noise)
30	0.9369
10	0.5314
3.333	0.2435
1.111	0.1025
0.37	0.0794
0.123	0.0287
0.041	0.0127
0	0

x= (y-0.0815)/ 0.0303



Appendix D10. Manufacturer's instructions for Human IL-6 ELISA kit.

SIGMA-ALDRICH™

sigma-aldrich.com

Certificate of Analysis

3050 Spruce Street, Saint Louis, MO 63103 USA
Tel: (800) 521-8956 (314) 771-5765 Fax: (800) 325-5052 (314) 771-5757

Product Name Human IL-6 ELISA Kit
for serum, plasma, and cell culture supernatants
Product Number RAB0306
Lot Number 0424F0140

Storage Store the kit at -20°C. It remains active for up to 1 year. Avoid repeated freeze-thaw cycles. The reconstituted standard should be stored at -20°C or -70°C (-70°C is recommended). Opened microplate strips or reagents may be stored for up to 1 month at 2-8°C. Return unused wells to the pouch containing desiccant pack and reseal along entire edge.

Components

1. Human IL-6 Antibody-coated ELISA Plate (Item A) - RABIL6A-EA: 96 wells (12 strips x 8 wells) coated with anti-Human IL-6.
2. 20x Wash Buffer (Item B) - RABWASH4
3. Lyophilized Human IL-6 Protein Standard (Item C) - RABMIL6S-1VL
4. Biotinylated Human IL-6 Detection Antibody (Item F) - RABIL6F-1VL
5. HRP-Streptavidin (Item G) - RABHRP5
6. ELISA Colorimetric TMB Reagent (HRP Substrate, Item H) - RABTMB3
7. ELISA Stop Solution (Item I) - RABSTOP3
8. ELISA 1x Assay/Sample Diluent Buffer A (Item D1) - RABELADA-30ML
9. ELISA 5x Assay/Sample Diluent Buffer B (Item E1) - RABELADB-15ML

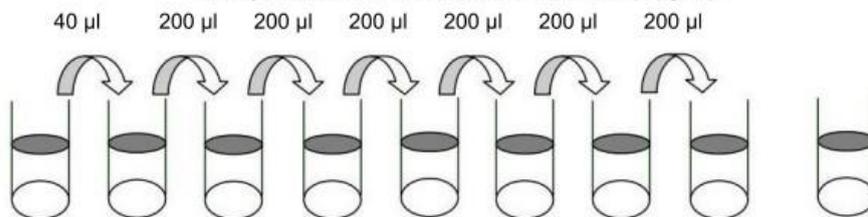
Assay/Sample Diluent Buffer dilution (Preparation, Step 2) Assay/Sample Diluent Buffer B (Item E1) should be diluted 5-fold with deionized or distilled water before use.

Sample Dilution (Preparation, Step 3) Assay/Sample Diluent Buffer A (Item D1) should be used for dilution of serum and plasma samples. 1x Assay/Sample Diluent Buffer B (Item E1) should be used for dilution of cell culture supernatant samples. The suggested dilution for normal serum/plasma is 2 fold.

* Please note that the levels of IL-6 may vary between different samples. Optimal dilution factors for each sample must be determined by the investigator.

**Preparation of Standard
(Preparation, Step 4)**

Briefly spin a vial of Item C. Add 500 µl Assay Diluent A (for serum/plasma samples) or 1X Assay Diluent B (for cell culture medium) into Item C vial to prepare a 12,000 pg/ml standard. Dissolve the powder thoroughly by a gentle mix. Add 40 µl IL-6 standard from the vial of Item C, into a tube with 440 µl Assay Diluent A or 1X Assay Diluent B to prepare a 1000 pg/ml standard solution. Pipette 400 µl Assay Diluent A or 1X Assay Diluent B into each tube. Use the 1000 pg/ml standard solution to produce a dilution series (Figure 1). Mix each tube thoroughly before the next transfer. Assay Diluent A or 1X Assay Diluent B serves as the zero standard (0 pg/ml).



		Std1	Std2	Std3	Std4	Std5	Std6	Std7		Zero Standard
Diluent volume	Item C+ 500 µl	440 µl	400 µl	400 µl	400 µl	400 µl	400 µl	400 µl		400 µl
Conc.	12,000 pg/ml	1000 pg/ml	333.3 pg/ml	111.1 pg/ml	37.04 pg/ml	12.35 pg/ml	4.12 pg/ml	1.37 pg/ml		0 pg/ml

**Preparation of Biotinylated
Detection Antibody
(Preparation, Step 6)**

Briefly spin the Detection Antibody vial (Item F) before use. Add 100 µl of 1x Diluent Buffer B (Item E1) into the vial to prepare a detection antibody concentrate. Pipette up and down to mix gently (the concentrate can be stored at 4°C for 5 days). The detection antibody concentrate should be diluted 80-fold with 1x Diluent Buffer B (Item E1) and used in Procedure, step 4.

**Dilution of HRP-Streptavidin
Concentrate
(Preparation, Step 7)**

Briefly spin the HRP-Streptavidin concentrate vial (Item G) and pipette up and down to mix gently before use, as precipitates may form during storage. HRP-Streptavidin concentrate should be diluted 600-fold with 1x Diluent Buffer B (Item E1).

For example: Briefly spin the vial (Item G) and pipette up and down to mix gently. Add 25 µl of HRP-Streptavidin concentrate into a tube with 15 ml 1X Assay Diluent B to prepare a final 600 fold diluted HRP-Streptavidin solution (don't store the diluted solution for next day use). Mix well.

Human IL-6 ELISA kit

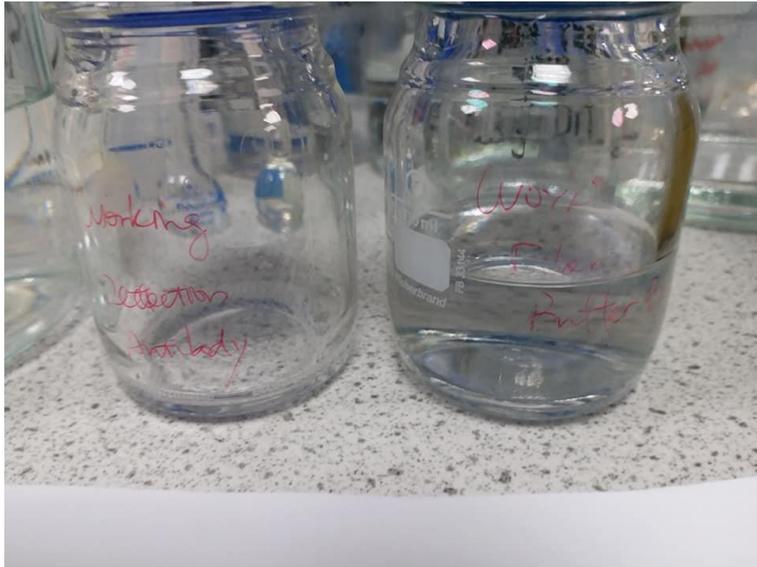
1.5ml or less each vial of blood plasma samples.



Bring all reagents and samples to room temperature (18–25 °C) before use.

Assay/sample Diluent buffer dilution = Streptavidin-HRP diluent:

1 volume of **sample diluent buffer B (Item E1)** with 4 volume of **deionized/distilled water** before use.
e.g. 15ml of **Item E1** ~ 60ml of deionized water = 75 ml in total of **Item E1**
Label as **Working Diluent Buffer B**



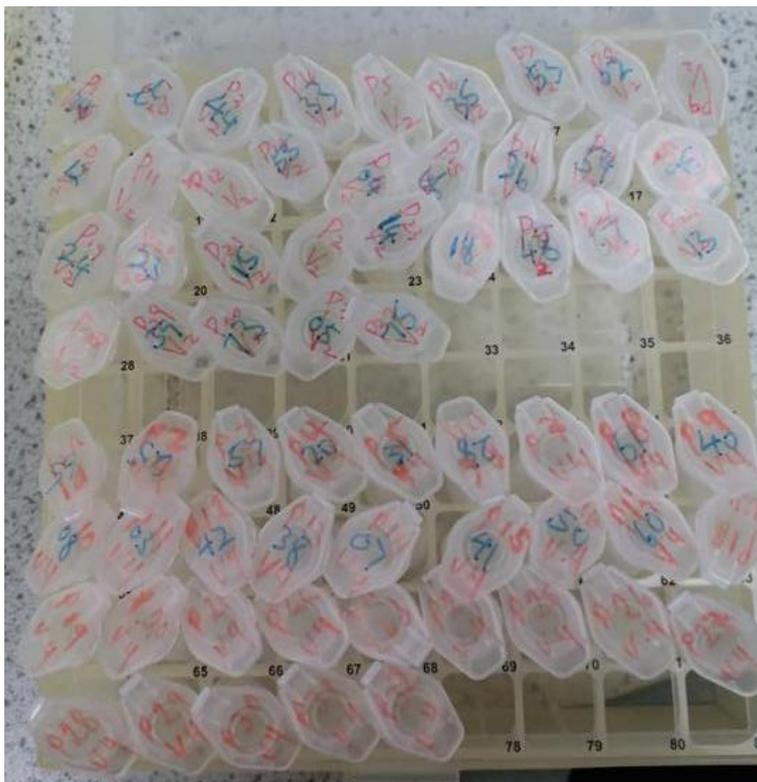
Sample diluent preparation:

Wearing gloves.

1 volume of sample with 2 fold volume of **standard diluent buffer A (Item D1).**

1 plasma with **(1 Item D1+ 1 plasma)**

IL-6 A suggested 2-fold dilution: 100ul / 100ul of sample + 100ul of Item D1



Preparation of Standard (for Procedure 2):

1. Spin 2 vials of **Human IL-6 Protein Standard (Item C)**;
2. Pipette **500 ul** reconstituted **Working Standard Diluent Buffer A (Item D1)** into 2 * Item C vial **(This makes the reconstituted standard in Item C vial; To prepare a 12000 pg/ml standard)**
3. Dissolve the powder thoroughly by a **Gentle Mix**.
4. Pipette **40 ul** reconstituted **IL-6 Protein standard** from Item C vial into a micro-tube with **440 ul** Item D1.

4. Prepare **8 tubes** labelled: **1000 pg/ml, 333.3, 111.1, 37.04, 12.35, 4.12, 1.37, 0.**
5. Pipette **200ul = 0.2 ml Standard Diluent Buffer A (Item D1)** into each **7 tubes** labelled as above.
6. Use the **1000 pg/ml standard solution** to produce a dilution series (**mix each tube thoroughly before the next transfer**).

	Standard:	Add:	Into:
1	12000 pg/ml (standard solution)	Describe above	
2	1000 pg/ml	0.04 ml of the 12000 pg/ml std.	0.44 ml of the diluent buffer A
3	333.3 pg/ml	0.2 ml of the 1000 pg/ml std.	0.4 ml of the diluent buffer A
4	111.1 pg/ml	0.2 ml of the 333.3 pg/ml std.	0.4 ml of the diluent buffer A
5	37.04 pg/ml	0.2 ml of the 111.1 pg/ml std.	0.4 ml of the diluent buffer A
6	12.35 pg/ml	0.2 ml of the 37.04 pg/ml std.	0.4 ml of the diluent buffer A
7	4.12 pg/ml	0.2 ml of the 12.35 pg/ml std.	0.4 ml of the diluent buffer A
8	0 pg/ml		0.4 ml of the diluent buffer A

Figure 1: *Sample Standard Diluent Buffer A (Item D1) serves as the zero standard (0 pg/ml)





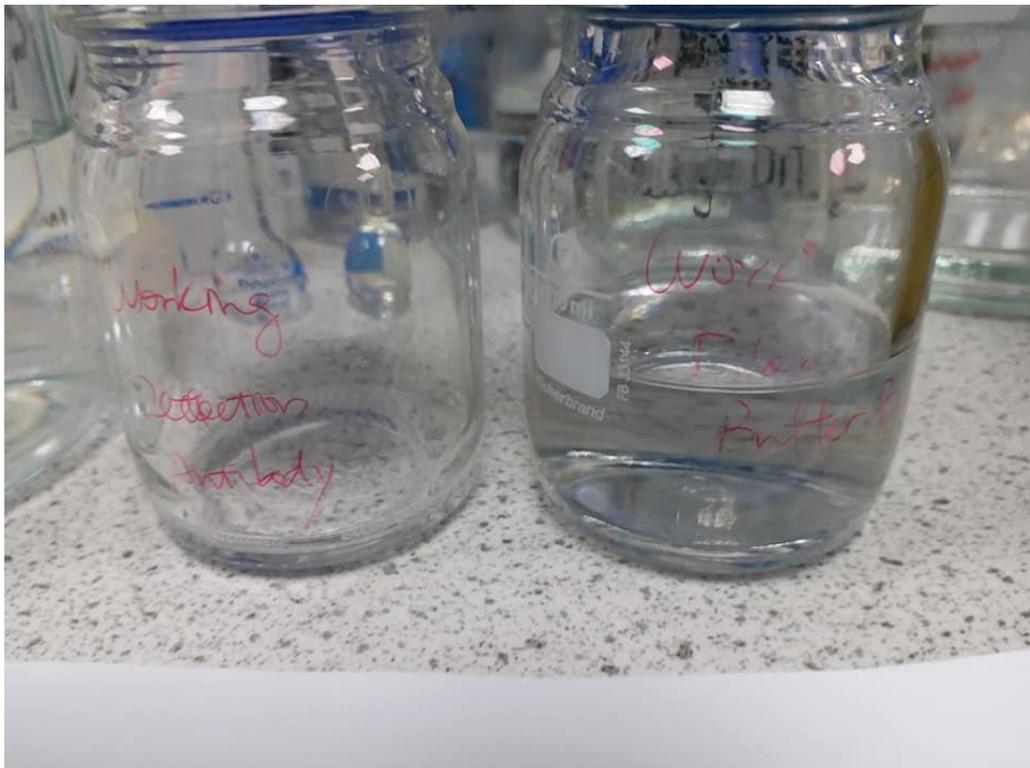
If the **Wash Buffer (20x) (Item B)** contains visible crystals, warm to room temperature and mix gently until any precipitated salts have dissolved. Dilute **25 ml** of Wash Buffer concentrate into **deionized or distilled water** to yield **500 ml of 1x Wash Buffer**.
 Dilute **1 volume** of the Wash Buffer Concentrate (20x) with **19 volumes** of deionized water.
 Add **25 ml** of the Wash Buffer concentrate (20x) into **475 ml** of deionized water.
 Label as **Working Wash Buffer**.





Preparation of Biotinylated Detection Antibody (Item F) (For Procedure 4):

1. Spin the **Item F vial** before use.
2. Add 100ul **Item E1** into the **Item F vial**.
 $1/80 = 100 / (x+100)$ $x = 7900$ ul **Item E1** $7900\text{ul} * 2 = 15.8\text{ml}$ of **Item E1**
Label as **Working Detection Antibody**.



Dilution of HRP-Streptavidin Concentrate (Item G) (For Procedure 6)

Spin **Item G**

Item G should be diluted **600 fold** with **1*Diluent Buffer B (Item E1)**

Pipette **25 ul** of **Item G** into a micro-tube with **15 ml** of **1*Item E1**, mix well.

Label as **Streptavidin-HRP working solution**.



Sandwich Assay Procedure (approx. 6 hours in total)

1. Bring all reagents and samples to room temperature (**18 - 25°C**) before use. Remove excess microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, and reseal.

It is recommended that all standards and samples be run at least in duplicate.

2. Add **100 µl** of each standard, sample or control into appropriate wells. Cover wells and incubate for **2.5 hours** at room temperature or overnight at 4 C with gentle shaking.

3. Thoroughly aspirate or decant the solution and wash **4 times** with Working Wash Buffer (**300ul**). Wash by filling each well with **Wash Buffer (300 µl)** using an auto-washer or a squirt bottle. Let soak for **15-30 seconds**, then aspirate the liquid 吸液, then repeat.



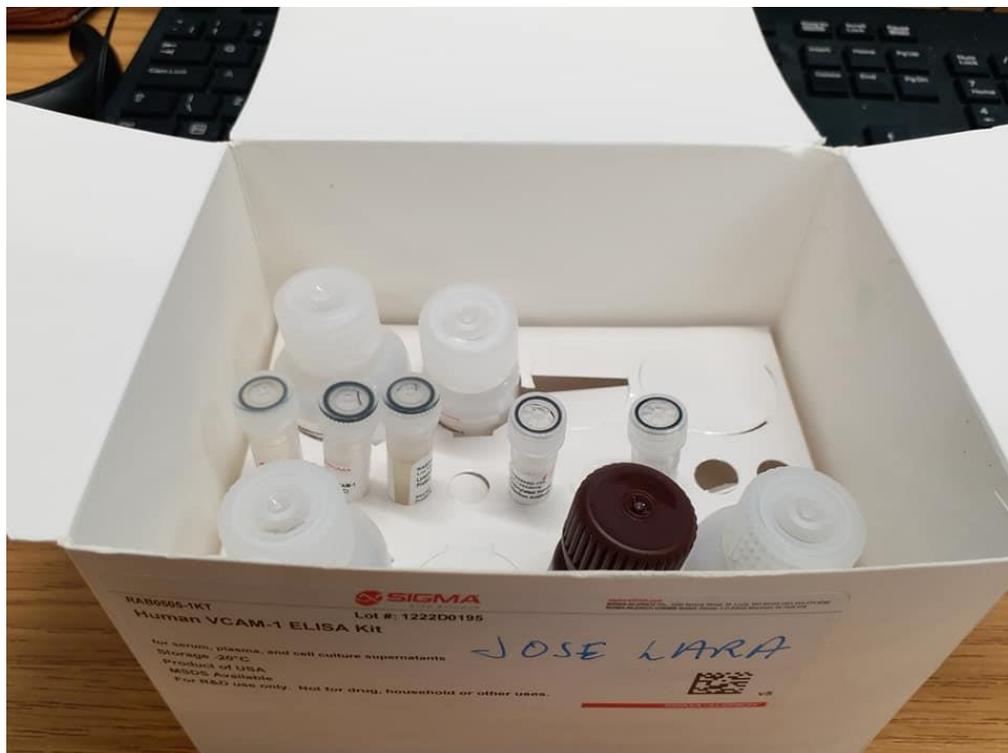
Squirt bottle. If squirt bottle is used, flood the plate with wash buffer, completely filling all wells. After the washing procedure, the plate is inverted, blot it against the clean paper towels 用干净的纸巾将其吸干 or and tapped dry on absorbent tissue.

Or we can see multi-pipette and tray to wash each well.

4. Pipette **100 µl** of 1x prepared **Detection Antibody = Biotinylated anti-(Biotin Conjugate)** to each well. Cover wells and incubate for **1 hour** at room temperature with gentle shaking.

5. Discard the solution. Repeat the wash procedure as in **step 3**.
6. Add **100 µl** of **prepared Streptavidin Working solution** to each well. Cover wells and incubate for **45 minutes** at room temperature with **gentle shaking**.
7. Thoroughly aspirate or decant solution from wells and discard the liquid. Wash wells 4 times. Repeat **step 3**.
8. Add **100 µl** of **TMB One-Step Substrate Reagent = Stabilized Chromogen = HRP Substrate (Item H)** to each well. Cover wells and incubate for **30 minutes** at room temperature **in the dark** with gentle shaking. The liquid in the wells will begin to turn **blue**. $100\ \mu\text{l} * (96-5)\ \text{wells} = 9.1\ \text{ml}$. $5\ \text{wells} * 100\ \mu\text{l} = 0.5\ \text{ml}$. $9.1\ \text{ml} / 96\ \text{wells} = 0.09\ \text{ml}$
Therefore, pipette 90 µl of HRP substrate into each well.
9. Add **50 µl** of **Stop Solution (Item I)** to each well. Read the absorbance of each well at **450 nm** immediately (**within 2 hours**) having blanked the plate reader against a chromogen blank composed of **substrate and stop solution**. $50\ \mu\text{l} * 91\ \text{wells} = 4.55\ \text{ml}$. $4.55\ \text{ml} / 96\ \text{wells} = 47\ \mu\text{l}$ for each well.
10. Determine the optical density of each well within **30 minutes**, using a microplate reader set to **450 nm** having blanked the plate reader against a chromogen blank composed of 100µl each of Stabilized Chromogen and stop solution.
If wavelength correction is available, set to 540 nm or 570 nm. If wavelength correction is not available, subtract readings at 540 nm or 570 nm from the readings at 450 nm. This subtraction will correct for optical imperfections in the plate. Readings made directly at 450 nm without correction may be higher and less accurate.

The minimum detectable dose of Human IL-6 was determined to be **3 pg/ml**.
Sample type: plasma; Average % Recovery: 93.74; Range (%): 84 - 104.



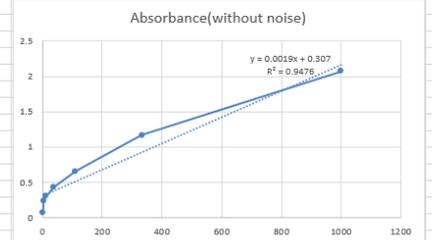


	1	2	3	4	5	6	7	8	9	10	11	12
A	2.1228	0.0721	0.075	0.0715	0.0747	0.0731	0.0783	0.1032	0.0886	0.1013	0.0707	0.0793
B	1.2186	0.0578	0.0866	0.0616	0.0661	0.0731	0.0976	0.0769	0.1194	0.1107	0.083	0.1877
C	0.7101	0.0936	0.0787	0.072	0.0605	0.081	0.1132	0.1383	0.1452	0.098	0.1033	0.1124
D	0.4817	0.0837	0.0608	0.0801	0.063	0.077	0.0665	0.0835	0.0762	0.1015	0.0768	0.087
E	0.3572	0.0739	0.0561	0.0736	0.0612	0.0599	0.0885	0.0756	0.0852	0.1059	0.0833	0.0804
F	0.2849	0.0906	0.0806	0.0751	0.1201	0.0823	0.0853	0.0808	0.0772	0.1189	0.0706	0.0808
G	0.1243	0.0805	0.0861	0.0817	0.0708	0.082	0.0815	0.0785	0.1145	0.083	0.1017	0.0687
H	0.0514	0.0671	0.1034	0.0749	0.0787	0.082	0.0625	0.0794	0.0998	0.0954	0.0725	0.0827

	Concentration (ng/ml)	Absorbance	Absorbance(without noise)
A	1000	2.1228	2.0714
B	333.3	1.2186	1.1672
C	111.1	0.7101	0.6587
D	37.04	0.4817	0.4303
E	12.35	0.3572	0.3058
F	4.12	0.2849	0.2335
G	1.37	0.1243	0.0729
H	0	0.0514	0

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	1	2	3	4	5	6	7	8	9	10	11	12
A	2.0714	0.0207	0.0236	0.0201	0.0233	0.0217	0.0269	0.0518	0.0372	0.0499	0.0193	0.0279
B	1.1672	0.0064	0.0352	0.0102	0.0147	0.0217	0.0462	0.0255	0.068	0.0593	0.0316	0.1363
C	0.6587	0.0422	0.0273	0.0206	0.0091	0.0296	0.0618	0.0869	0.0938	0.0466	0.0519	0.061
D	0.4303	0.0323	0.0094	0.0287	0.0116	0.0256	0.0151	0.0321	0.0248	0.0501	0.0254	0.0356
E	0.3058	0.0225	0.0047	0.0222	0.0098	0.0085	0.0371	0.0242	0.0338	0.0545	0.0319	0.029
F	0.2335	0.0392	0.0292	0.0237	0.0687	0.0309	0.0339	0.0294	0.0258	0.0675	0.0192	0.0294
G	0.0729	0.0291	0.0347	0.0303	0.0194	0.0306	0.0301	0.0271	0.0631	0.0316	0.0503	0.0173
H	0	0.0157	0.052	0.0235	0.0273	0.0306	0.0111	0.028	0.0484	0.044	0.0211	0.0313



$$x=(y-0.307)/0.0019$$

	1	2	3	4	5	6	7	8	9	10	11	12
A	1000	150.684	149.158	151	149.316	150.158	147.421	134.316	142	135.316	151.421	146.895
B	333.3	158.211	143.053	156.211	153.842	150.158	137.263	148.158	125.789	130.368	144.947	89.8421
C	111.1	139.368	147.211	150.737	156.789	146	129.053	115.842	112.211	137.053	134.263	129.474
D	37.04	144.579	156.632	146.474	155.474	148.105	153.632	144.684	148.526	135.211	148.211	142.842
E	12.35	149.737	159.105	149.895	156.421	157.105	142.053	148.842	143.789	132.895	144.789	146.316
F	4.12	140.947	146.211	149.105	125.421	145.316	143.737	146.105	148	126.053	151.474	146.105
G	1.37	146.263	143.316	145.632	151.368	145.474	145.737	147.316	128.368	144.947	135.105	152.474
H	0	153.316	134.211	149.211	147.211	145.474	155.737	146.842	136.105	138.421	150.474	145.105

	1	2	3	4	5	6	7	8	9	10	11	12
A	1000	301.368	298.316	302	298.632	300.316	294.842	268.632	284	270.632	302.842	293.789
B	333.3	316.421	286.105	312.421	307.684	300.316	274.526	296.316	251.579	260.737	289.895	179.684
C	111.1	278.737	294.421	301.474	313.579	292	258.105	231.684	224.421	274.105	268.526	258.947
D	37.04	289.158	313.263	292.947	310.947	296.211	307.263	289.368	297.053	270.421	296.421	285.684
E	12.35	299.474	318.211	299.789	312.842	314.211	284.105	297.684	287.579	265.789	289.579	292.632
F	4.12	281.895	292.421	298.211	250.842	290.632	287.474	292.211	296	252.105	302.947	292.211
G	1.37	292.526	286.632	291.263	302.737	290.947	291.474	294.632	256.737	289.895	270.211	304.947
H	0	306.632	268.421	298.421	294.421	290.947	311.474	293.684	272.211	276.842	300.947	290.211

Appendix D11. Manufacturer's instructions for Human PAI-1 ELISA kit.

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Certificate of Analysis

3050 Spruce Street, Saint Louis, MO 63103 USA
Tel: (800) 521-8956 (314) 771-5765 Fax: (800) 325-5052 (314) 771-5757

Product Name Human PAI-I ELISA Kit
for serum, plasma, and cell culture supernatants
Product Number RAB0429
Lot Number 1016F0312

Storage Store the kit at -20°C. It remains active for up to 1 year. Avoid repeated freeze-thaw cycles. The reconstituted standard should be stored at -20°C or -70°C (-70°C is recommended). Opened microplate strips or reagents may be stored for up to 1 month at 2-8°C. Return unused wells to the pouch containing desiccant pack and reseal along entire edge.

Components

1. Human PAI-1 Antibody-coated ELISA Plate (Item A) - RAB0429A-EA: 96 wells (12 strips x 8 wells) coated with anti-Human PAI-1.
2. 20x Wash Buffer (Item B) - RABWASH4
3. Lyophilized Human PAI-1 Protein Standard (Item C) - RAB0429C-1VL
4. Biotinylated Human PAI-1 Detection Antibody (Item F) - RAB0429D-1VL
5. HRP-Streptavidin (Item G) - RABHRP5
6. ELISA Colorimetric TMB Reagent (HRP Substrate, Item H) - RABTMB3
7. ELISA Stop Solution (Item I) - RABSTOP3
8. ELISA 1x Assay/Sample Diluent Buffer A (Item D1) - RABELADA-30ML
9. ELISA 5x Assay/Sample Diluent Buffer B (Item E1) - RABELADB-15ML

Assay/Sample Diluent Buffer dilution (Preparation, Step 2)

Assay/Sample Diluent Buffer B (Item E1) should be diluted 5-fold with deionized or distilled water before use.

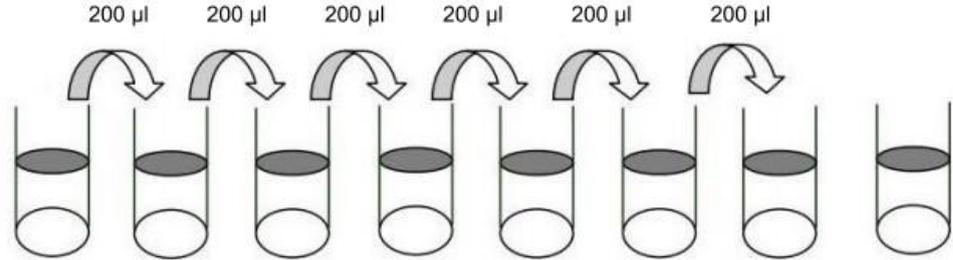
Sample Dilution (Preparation, Step 3)

Assay/Sample Diluent Buffer A (Item D1) should be used for dilution of serum and plasma samples. 1x Assay/Sample Diluent Buffer B (Item E1) should be used for dilution of cell culture supernatant samples. The suggested dilution for normal serum/plasma is 20 - 200 fold.

* Please note that the levels of PAI-1 may vary between different samples. Optimal dilution factors for each sample must be determined by the investigator.

**Preparation of Standard
(Preparation, Step 4)**

Briefly spin a vial of Item C. Add 800 µl Assay Diluent A (for serum/plasma samples) or 1X Assay Diluent B (for cell culture supernatants) into Item C vial to prepare a 25 ng/ml standard. Dissolve the powder thoroughly by a gentle mix. Pipette 300 µl Assay Diluent A or 1X Assay Diluent B into each tube. Use the 25 ng/ml standard solution to produce a dilution series (Figure 1). Mix each tube thoroughly before the next transfer. Assay Diluent A or 1X Assay Diluent B serves as the zero standard (0 ng/ml).



	Std1	Std2	Std3	Std4	Std5	Std6	Std7		Zero Standard
Diluent volume	Item C + 800 µl	300 µl	300 µl	300 µl	300 µl	300 µl	300 µl		300 µl
Conc.	25 ng/ml	10 ng/ml	4 ng/ml	1.6 ng/ml	0.64 ng/ml	0.256 ng/ml	0.102 ng/ml		0 ng/ml

**Preparation of Biotinylated Detection Antibody
(Preparation, Step 6)**

Briefly spin the Detection Antibody vial (Item F) before use. Add 100 µl of 1x Diluent Buffer B (Item E1) into the vial to prepare a detection antibody concentrate. Pipette up and down to mix gently (the concentrate can be stored at 4°C for 5 days). The detection antibody concentrate should be diluted 80-fold with 1x Diluent Buffer B (Item E1) and used in Procedure, step 4.

**Dilution of HRP-Streptavidin Concentrate
(Preparation, Step 7)**

Briefly spin the HRP-Streptavidin concentrate vial (Item G) and pipette up and down to mix gently before use, as precipitates may form during storage. HRP-Streptavidin concentrate should be diluted 400-fold with 1x Diluent Buffer B (Item E1).

For example: Briefly spin the vial (Item G) and pipette up and down to mix gently. Add 25 µl of HRP-Streptavidin concentrate into a tube with 10 ml 1X Assay Diluent B to prepare a final 400 fold diluted HRP- Streptavidin solution (don't store the diluted solution for next day use). Mix well.

2019-11-19 17:08:52

1	2	3	4	5	6	7	8	9	10	11	12
0.9425	1.0611	1.0966	0.7583	0.7632	0.9848	0.7914	0.4359	0.2595	1.8232	1.0746	1.2471
0.5648	1.4218	0.5608	0.8128	1.0356	0.3285	0.6871	0.4024	0.9364	1.1799	0.3757	0.879
0.3773	0.9733	1.0565	1.1096	0.6911	1.0432	1.0096	0.9703	0.6167	1.472	0.2964	1.2517
0.2879	0.7941	0.6249	0.7701	1.4698	0.8266	0.6227	0.5343	0.9391	1.5711	0.6632	0.7
0.2384	0.6234	0.8868	0.7806	0.6794	0.7075	0.458	0.5006	0.5155	0.8333	0.6461	1
0.2268	0.9437	0.874	0.4856	1.5327	0.2306	0.5824	0.4287	0.4825	0.9386	0.564	0.4202
0.1189	1.0319	0.8232	1.0932	0.8072	0.5014	0.4275	0.4382	0.8222	1.2851	1.2884	0.5879
0	0.3698	0.8481	0.6058	0.6455	0.4015	0.5247	0.3872	0.432	1.2822	0.6201	0.376

$x=(y-0.1494)/0.0785$

1	2	3	4	5	6	7	8	9	10	11	12
10	11.61401	12.06624	7.756688	7.819108	10.64204	8.178344	3.649682	1.402548	21.32229	11.78599	13.98344
4	16.20892	5.240764	8.450955	11.28917	2.281529	6.849682	3.22293	10.02548	13.12739	2.882803	9.294268
1.6	10.49554	11.55541	12.23185	6.900637	11.38599	10.95796	10.45732	5.952866	16.84841	1.872611	14.04204
0.64	8.212739	6.057325	7.907006	16.82038	8.626752	6.029299	4.903185	10.05987	18.11083	6.545223	7.014013
0.256	6.038217	9.393631	8.040764	6.751592	7.109554	3.93121	4.473885	4.663694	8.712102	6.327389	10.83567
0.102	10.11847	9.230573	4.282803	17.62166	1.034395	5.515924	3.557962	4.243312	10.0535	5.281529	3.449682
0.041	11.24204	8.583439	12.02293	8.379618	4.484076	3.542675	3.678991	8.570701	14.46752	14.50955	5.585987
0	2.807643	8.900637	5.814013	6.319745	3.211465	4.780892	3.029299	3.6	14.43057	5.996178	2.886624

$x=(y-0.1494)/0.0785$

	Concentration (ng/ml)	Absorbance	Absorbance (without noise)
A	10	1.0819	0.9425
B	4	0.7042	0.5648
C	1.6	0.5167	0.3773
D	0.64	0.4273	0.2879
E	0.256	0.3778	0.2384
F	0.102	0.3662	0.2268
G	0.041	0.2583	0.1189
H	0	0.1394	0

1	2	3	4	5	6	7	8	9	10	11	12
10	232.2803	241.3248	155.1338	156.3822	212.8408	163.5669	72.99363	28.05096	426.4459	235.7197	279.6688
4	324.1783	104.8153	169.0191	225.7834	45.63057	136.9936	64.4586	200.5096	262.5478	57.65605	185.8854
1.6	209.9108	231.1083	244.6369	138.0127	227.7197	219.1592	209.1465	119.0573	336.9682	37.45223	280.8408
0.64	164.2548	121.1465	158.1401	336.4076	172.535	120.586	98.06369	201.1975	362.2166	130.9045	140.2803
0.256	120.7643	187.8726	160.8153	135.0318	142.1911	78.6242	89.47771	93.27389	174.242	126.5478	216.7134
0.102	202.3694	184.6115	85.65605	352.4331	20.6879	110.3185	71.15924	84.86624	201.0701	105.6306	68.99363
0.041	224.8408	171.6688	240.4586	167.5924	89.68153	70.8535	73.57962	171.414	289.3503	290.1911	111.7197
0	56.15287	178.0127	116.2803	126.3949	64.2293	95.61783	60.58599	72	288.6115	119.9236	57.73248

Appendix D12. Manufacturer's instructions for Human CRP ELISA kit.



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Certificate of Analysis / Protocol 3050 Spruce Street, Saint Louis, MO 63103 USA
Tel: (800) 521-8956 (314) 771-5765 Fax: (800) 325-5052 (314) 771-5757

Product Name Human C-Reactive Protein ELISA Kit
for serum, plasma, and cell culture supernatants
Product Number RAB0096
Lot Number 0925F0283

Storage Store the kit at -20°C. It remains active for up to 1 year. Avoid repeated freeze-thaw cycles. The reconstituted standard should be stored at -20°C or -70°C (-70°C is recommended). Opened microplate strips or reagents may be stored for up to 1 month at 2-8°C. Return unused wells to the pouch containing desiccant pack and reseal along entire edge.

Components

1. Human CRP Antibody-coated ELISA Plate (Item A) - RAB0096A-EA: 96 wells (12 strips x 8 wells) coated with anti-Human CRP.
2. 20x Wash Buffer (Item B) - RABWASH4
3. Lyophilized Human CRP Protein Standard (Item C) - RAB0096C-1VL
4. Biotinylated Human CRP Detection Antibody (Item F) - RAB0096D-1VL
5. HRP-Streptavidin (Item G) - RABHRP5
6. ELISA Colorimetric TMB Reagent (HRP Substrate, Item H) - RABTMB3
7. ELISA Stop Solution (Item I) - RABSTOP3
8. ELISA 5x Assay/Sample Diluent Buffer D (Item K) - RABELADD-15ML (2 bottles)
9. ELISA 5x Assay/Sample Diluent Buffer B (Item E1) - RABELADB-15ML

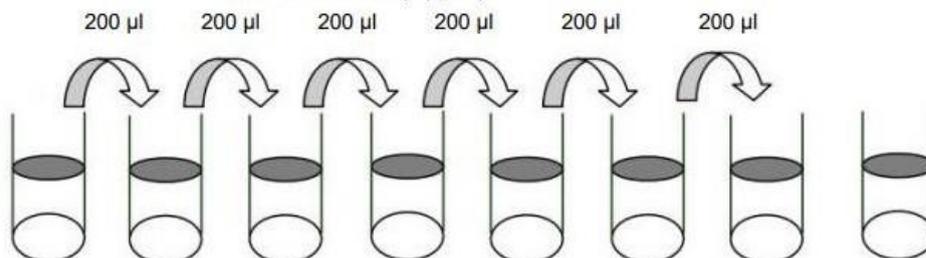
Assay/Sample Diluent Buffer dilution (Preparation, Step 2) Assay/Sample Diluent Buffers B (Item E1) and D (Item K) should be diluted 5-fold with deionized or distilled water before use.

Sample Dilution (Preparation, Step 3) 1x Assay/Sample Diluent Buffer D (Item K) should be used for dilution of serum, plasma, and cell culture supernatant samples. The suggested dilution for normal serum/plasma is 20,000 fold. For example, add 2 µl of serum/plasma into a tube with 398.0 µl 1X Assay Diluent D to prepare a 200-fold diluted sample. Mix thoroughly and then pipette 3 µl of prepared 200-fold diluted sample into a tube with 297 µl 1X Assay Diluent D to prepare a final 20,000 fold diluted sample.

* Please note that the levels of CRP may vary between different samples. Optimal dilution factors for each sample must be determined by the investigator.

**Preparation of Standard
(Preparation, Step 4)**

Briefly spin a vial of Item C. Add 1,200 μ l 1X Assay Diluent D (Item K) into Item C vial to prepare a 25,000 pg/ml standard solution. Dissolve the powder thoroughly by a gentle mix. Pipette 400 μ l 1X Assay Diluent D into each tube. Use the stock standard solution to produce a dilution series (Figure 1). Mix each tube thoroughly before the next transfer. 1X Assay Diluent D serves as the zero standard (0 pg/ml).



	Std1	Std2	Std3	Std4	Std5	Std6	Std7		Zero Standard
Diluent volume	Item C + 1,200 μ l	400 μ l		400 μ l					
Conc.	25000 pg/ml	8333 pg/ml	2778 pg/ml	925.9 pg/ml	308.6 pg/ml	102.9 pg/ml	34.29 pg/ml		0 pg/ml

**Preparation of Biotinylated
Detection Antibody
(Preparation, Step 6)**

Briefly spin the Detection Antibody vial (Item F) before use. Add 100 μ l of 1x Diluent Buffer B (Item E1) into the vial to prepare a detection antibody concentrate. Pipette up and down to mix gently (the concentrate can be stored at 4°C for 5 days). The detection antibody concentrate should be diluted 80-fold with 1x Diluent Buffer B (Item E1) and used in Procedure, step 4.

**Dilution of HRP-Streptavidin
Concentrate
(Preparation, Step 7)**

Briefly spin the HRP-Streptavidin concentrate vial (Item G) and pipette up and down to mix gently before use, as precipitates may form during storage. HRP-Streptavidin concentrate should be diluted 300-fold with 1x Diluent Buffer B (Item E1).

For example: Briefly spin the vial (Item G) and pipette up and down to mix gently. Add 40 μ l of HRP-Streptavidin concentrate into a tube with 12 ml 1X Assay Diluent B to prepare a final 300 fold diluted HRP-Streptavidin solution (don't store the diluted solution for next day use). Mix well.

Sigma-Aldrich CRP ELISA kit Procedure (approx. 6 hours)

Bring all reagents and samples to room temperature (18–25 °C) before use. Samples and ELISA kit need to take out before use 8 hours.

Sample diluent preparation:

- 1 volume of sample diluent buffer D (Item K) with 4 volume of deionized water.
15ml ~ 60ml = 75 ml of buffer D in total
2. Pipette 2 µl of plasma into a tube with 398.0 µl 1X Assay Diluent D (Item K) to prepare a 200-fold diluted sample.
3. Mix thoroughly
4. Pipette 3 µl of prepared 200-fold diluted sample into a tube with 297 µl 1X Assay Diluent D (Item K) to prepare a final 20,000- fold diluted sample.
(398+297) ul * 88 samples = 61160 ul = 61.16ml

Preparation of Standard:

1. Spin 2 vials of Human CRP Protein Standard (Item C):
2. Pipette 1200 ul Assay Diluent D (Item K) into 2 * Item C vial
3. Gentle Mix.
4. Pipette 400 ul Assay Diluent D (Item K) into each 8 labelled tube
5. Use the 25,000 pg/ml standard solution to produce a dilution series (mix each tube thoroughly before the next transfer).

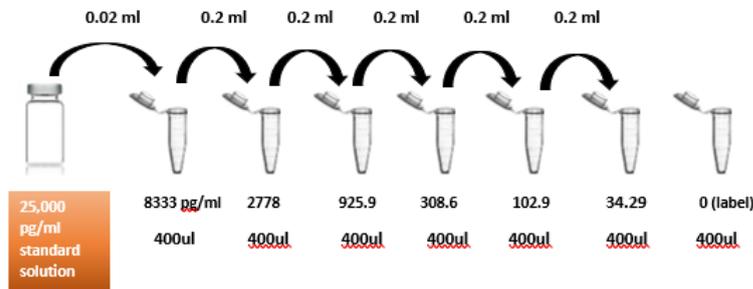


fan.liang
This makes the reconstituted standard in Item C vial
To prepare a 25,000 pg/ml standard solution

1200ul * 2 = 2400 ul
400ul * 8 = 3200 ul
5.6ml

	Standard:	Add:	Into:
1	25000 (standard solution)		Describe above
2	8333	0.2 ml of the 25000 pg/ml std.	0.4 ml of the diluent D
3	2778	0.2 ml of the 8333 pg/ml std.	0.4 ml of the diluent D
4	925.9	0.2 ml of the 2778 pg/ml std.	0.4 ml of the diluent D
5	308.6	0.2 ml of the 925.9 pg/ml std.	0.4 ml of the diluent D
6	102.9	0.2 ml of the 308.6 pg/ml std.	0.4 ml of the diluent D
7	34.29	0.2 ml of the 102.9 pg/ml std.	0.4 ml of the diluent D
8	0		0.4 ml of the diluent D

Figure 1: *Assay Diluent D (Item K) serves as the zero standard (0 pg/ml)



Add **100 µl** of each standard, sample or control into appropriate wells. Cover wells and incubate for **2.5 hours** at room temperature or overnight at 4 C **with gentle shaking**.



fan.liang
E319, put in TECAN SPARK 10M to gently shake.

Assay/sample Diluent buffer B dilution = Streptavidin-HRP diluent:

1 volume of sample diluent buffer B (Item E1) with 4 volume of deionized/distilled water before use.

e.g. 15ml of Item E1 ~ 60ml of deionized water = 75 ml in total of Item E1

Label as Working Diluent Buffer B

If the Wash Buffer (20x) (Item B) contains visible crystals, warm to room temperature and mix gently until any precipitated salts have dissolved. Dilute 25 ml of Wash Buffer concentrate into deionized or distilled water to yield **500 ml of 1x Wash Buffer**.

Add 25 ml of the Wash Buffer concentrate (20x) into 475 ml of deionized water.

Label as Working Wash Buffer.



fan.liang
Store both concentrate and the working wash buffer in the refrigerator, the dilute buffer should be used within 14 days.

Preparation of Biotinylated Detection Antibody (Item F):

1. Spin Item F vial.

2. Add 100ul Diluent B Item E1 into the Item F vial.

3. Pipette up and down to mix gently (the concentrate can be stored at 4 for 5 days).

$1/80 = 100 / (x+100) \quad x = 7900 \text{ ul Item E1} * 2 = 15.8\text{ml}$

Label as Working Detection Antibody.



fan.liang
The detection antibody concentrate should be diluted 80 fold with 1* Diluent Buffer B (Item E1)

Dilution of HRP-Streptavidin Concentrate (Item G)

Spin Item G – pipette up and down to mix gently before use, as precipitates may form during storage.

Item G should be diluted **300 fold** with 1*Diluent Buffer B (Item E1)

Pipette 40 ul of Item G into a tube with 12 ml of 1*Item E1, mix well.

Label as Streptavidin-HRP working solution.



fan.liang
Within 15 minutes of usage



fan.liang
To prepare a final 300-fold diluted HRP-Streptavidin solution

Thoroughly aspirate or decant the solution and wash 3 times with Working Wash Buffer (300ul).

Wash by filling each well with Wash Buffer (300 µl) using an auto-washer or a squirt bottle. Let soak for **15-30 seconds**, then aspirate the liquid 吸液 then repeat.



fan.liang
 $300 \text{ ul} * 96 \text{ wells} = 28.8 \text{ ml}$
 $28.8 * 3 = 86.4\text{ml}$
 $500 \text{ ml} / 3 = 166 \text{ ml}$ each time of washing
 $166\text{ml} / 3 = 55 \text{ ml}$

Squirt bottle. If squirt bottle is used, flood the plate with wash buffer, completely filling all wells. After the washing procedure, the plate is inverted, blot it against the clean paper towels **用干净的纸巾将其吸干** or and tapped dry on absorbent tissue. Or use the multi-pipette and tray

Pipette **100 µl** of 1x prepared Detection Antibody = Biotinylated anti-(Biotin Conjugate) to each well. Cover wells and incubate for **1 hour** at room temperature with **gentle shaking**.

Thoroughly aspirate or decant solution from wells and discard the liquid.

Add **100 µl** of prepared Streptavidin Working solution to each well. Cover wells and incubate for **45 minutes** at room temperature with **gentle shaking**.

Thoroughly aspirate or decant solution from wells and discard the liquid.

Add **100 µl** of TMB One-Step Substrate Reagent = Stabilized Chromogen = HRP Substrate (Item H) to each well. Cover wells and incubate for **30 minutes** at room temperature **in the dark** with gentle shaking. The liquid in the wells will begin to turn **blue**.

Add **50 µl** of Stop Solution (Item I) to each well. Read the absorbance of each well at **450 nm** immediately (**within 2 hours**) having blanked the plate reader against a chromogen blank composed of substrate and stop solution.

Determine the optical density of each well within **30 minutes**, using a microplate reader set to **450 nm** having blanked the plate reader against a chromogen blank composed of 100ul each of Stabilized Chromogen and stop solution. The minimum detectable dose of Human CRP was determined to be **34 µg/ml**.

Results

Calculate the mean absorbance for each set of duplicate standards, controls, and samples, and subtract the average zero standard optical density. Plot the standard curve using **SigmaPlot** software, with standard concentration on the x-axis and absorbance on the y-axis. Draw the best-fit curve through the standard points.

Yellow: visit 2; Green: visit 4; Grapefruit: visit 1; Gray: visit 3

Data Templates

	1	2	3	4	5	6	7	8	9	10	11	12
A	1000	P1V2	P9 V2	P17 V2	P25 V2	P1V4	P9 V4	P17 V4	P25 V4	P1V1	P21V1	P5V3
B	333.3	P2V2	P10 V2	P18 V2	P26 V2	P2V4	P10 V4	P18 V4	P26 V4	P2V1	P22V1	P6V3
C	111.1	P3V2	P11 V2	P19 V2	P27 V2	P3V4	P11 V4	P19 V4	P27 V4	P3V1	P23V1	P19V3
D	37.04	P4V2	P12 V2	P20 V2	P28 V2	P4V4	P12 V4	P20 V4	P28 V4	P4V1	P24V1	P20V3
E	12.35	P5V2	P13 V2	P21 V2	P29 V2	P5V4	P13 V4	P21 V4	P29 V4	P5V1	P1V3	P21V3
F	4.12	P6V2	P14 V2	P22 V2	P30 V2	P6V4	P14 V4	P22 V4	P30 V4	P6V1	P2V3	P22V3
G	1.37	P7V2	P15 V2	P23 V2	P31 V2	P7V4	P15 V4	P23 V4	P31 V4	P19V1	P3V3	P23V3
H	0	P8V2	P16 V2	P24 V2	P32 V2	P8V4	P16 V4	P24 V4	P32 V4	P20V1	P4V3	P24V3

#8-well strips CB: Chromogen blank 色原空白

	1	2	3	4	5	6	7	8	9	10	11	12
A	1000	MM2	LuP2	STC2	ZJN2	MM4	LuP 4	STC 4	ZJN 4	MM1	LWD1	AD3
B	333.3	FG2	NN2	AH2	JR2	FG 4	NN 4	AH 4	JR 4	FG1	WL1	RD3
C	111.1	YY2	MC2	DDN2	JC2	YY 4	MC 4	DDN 4	JC 4	YY1	AW1	DDN3
D	37.04	JH2	JHE2	KTC2	RHB2	JH 4	JHE 4	KTC 4	RHB 4	JH1	HMC1	KTC3
E	12.35	AD2	TS2	LWD2	Y22	AD 4	TS 4	LWD 4	YZ 4	AD1	MM3	LWD3
F	4.12	RD2	IJ2	WL2	BRR2	RD 4	IJ 4	WL 4	BRR 4	RD1	FG3	WL3
G	1.37	JBT2	SC2	AW2	XL2	JBT 4	SC 4	AW 4	XL 4	DDN1	YY3	AW3
H	0	SS2	JJ2	HMC2	RH2	SS 4	JJ 4	HMC 4	RH 4	KTC1	JH3	HMC3

fan.liang
30 mins in the dark
**Do not cover the plate with aluminium foil or metalized mylar.*
100 µl * (96-5) wells = 9.1 ml.
5 wells * 100 µl = 0.5 ml.
9.1 ml / 96 wells = 0.09 ml

Therefore, pipette 90 µl of HRP substrate into each well.

fan.liang
The color in the wells should change from blue to yellow. If the color in the wells is green or the color change does not appear uniform, gently tap the plate to ensure thorough mixing.

50 µl * 91 wells = 4.55 ml.
4.55 ml / 96 wells = 47 µl for each well.

fan.liang
I will suggest that you include 12 samples from visit 1 and 12 samples from visit 3. These samples should belong to the same participants (for example participant 1, 2,3,4, etc for visit one, and also participants 1,2,3,4, etc for visit 3).

So, we will have 32 samples for visit 2, 32 samples for visit 4, 12 samples for visit 1, 12 samples for visit 3, and 8 standard curve. Total of 96, which is the number of wells we have in a microplate.

Appendix D13. Manufacturer's instructions for Human sVCAM-1 ELISA kit.

SIGMA-ALDRICH™

sigma-aldrich.com

Certificate of Analysis 3050 Spruce Street, Saint Louis, MO 63103 USA
Tel: (800) 521-8956 (314) 771-5765 Fax: (800) 325-5052 (314) 771-5757

Product Name Human VCAM-1 ELISA Kit
for serum, plasma, and cell culture supernatants

Product Number RAB0505

Lot Number 1222D0195

Storage Store the kit at -20°C. It remains active for up to 1 year. Avoid repeated freeze-thaw cycles. The reconstituted standard should be stored at -20°C or -70°C (-70°C is recommended). Opened microplate strips or reagents may be stored for up to 1 month at 2-8°C. Return unused wells to the pouch containing desiccant pack and reseal along entire edge.

Components

1. Human VCAM-1 Antibody-coated ELISA Plate (Item A) - RAB0505A-EA: 96 wells (12 strips x 8 wells) coated with anti-Human VCAM-1.
2. 20x Wash Buffer (Item B) - RABWASH4
3. Lyophilized Human VCAM-1 Protein Standard (Item C) - RAB0505C-1VL
4. Biotinylated Human VCAM-1 Detection Antibody (Item F) - RAB0505D-1VL
5. HRP-Streptavidin (Item G) - RABHRP5
6. ELISA Colorimetric TMB Reagent (HRP Substrate, Item H) - RABTMB3
7. ELISA Stop Solution (Item I) - RABSTOP3
8. ELISA 1x Assay/Sample Diluent Buffer A (Item D1) - RABELADA-30ML
9. ELISA 5x Assay/Sample Diluent Buffer B (Item E1) - RABELADB-15ML

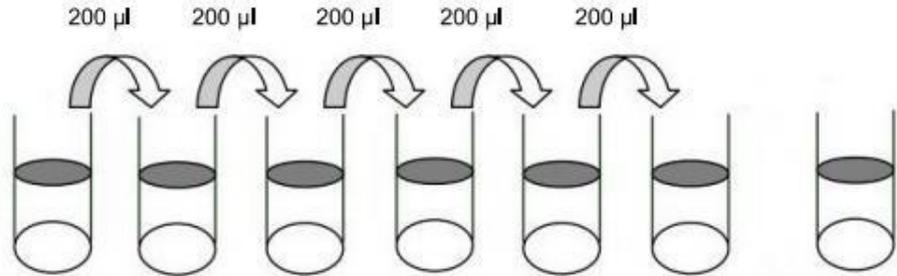
Assay/Sample Diluent Buffer dilution (Preparation, Step 2) Assay/Sample Diluent Buffer B (Item E1) should be diluted 5-fold with deionized or distilled water before use.

Sample Dilution (Preparation, Step 3) Assay/Sample Diluent Buffer A (Item D1) should be used for dilution of serum and plasma samples. 1x Assay/Sample Diluent Buffer B (Item E1) should be used for dilution of cell culture supernatant samples. The suggested dilution for normal serum/plasma is 10 - 50 fold.

* Please note that the levels of VCAM-1 may vary between different samples. Optimal dilution factors for each sample must be determined by the investigator.

**Preparation of Standard
(Preparation, Step 4)**

Briefly spin a vial of Item C. Add 600 µl Assay Diluent A (for serum/plasma samples) or 1X Assay Diluent B (for cell culture medium) into Item C vial to prepare a 60 ng/ml standard, Dissolve the powder thoroughly by a gentle mix. Pipette 400 µl Assay Diluent A or 1X Assay Diluent B into each tube. Use the 60 ng/ml standard solution to produce a dilution series (Figure 1). Mix each tube thoroughly before the next transfer. Assay Diluent A or 1X Assay Diluent B serves as the zero standard (0 ng/ml).



	Std1	Std2	Std3	Std4	Std5	Std6	Zero Standard
Diluent volume	Item C + 600 µl	400 µl	400 µl	400 µl	400 µl	400 µl	400 µl
Conc.	60 ng/ml	20 ng/ml	6.667 ng/ml	2.222 ng/ml	0.741 ng/ml	0.247 ng/ml	0 ng/ml

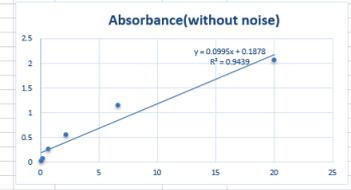
**Preparation of Biotinylated
Detection Antibody
(Preparation, Step 6)**

Briefly spin the Detection Antibody vial (Item F) before use. Add 100 µl of 1x Diluent Buffer B (Item E1) into the vial to prepare a detection antibody concentrate. Pipette up and down to mix gently (the concentrate can be stored at 4°C for 5 days). The detection antibody concentrate should be diluted 65-fold with 1x Diluent Buffer B (Item E1) and used in Procedure, step 4.

**Dilution of HRP-Streptavidin
Concentrate
(Preparation, Step 7)**

Briefly spin the HRP-Streptavidin concentrate vial (Item G) and pipette up and down to mix gently before use, as precipitates may form during storage. HRP-Streptavidin concentrate should be diluted 320-fold with 1x Diluent Buffer B (Item E1).
For example: Briefly spin the vial (Item G) and pipette up and down to mix gently. Add 37.5 µl of HRP-Streptavidin concentrate into a tube with 12 ml 1X Assay Diluent B to prepare a final 320 fold diluted HRP- Streptavidin solution.

	1	2	3	4	5	6	7	8	9	10	11	12	
A	2.3933	60	9.64522613	11.038191	7.24321608	12.0351759	3.70050251	8.06130653	7.67537688	4.65226131	4.70251256	0.614070352	4.30854271
B	2.0636	20	7.54472362	12.6884422	6.421105528	8.50452261	9.00301508	6.1839196	3.55678392	3.80904523	5.30452261	7.410050251	6.23819095
C	1.537	6.667	17.6030151	7.91256281	6.425125628	10.4201005	11.2633166	8.63115578	6.95879397	9.32763819	5.43819095	2.267336683	2.62914573
D	0.5481	2.222	18.2532663	11.4874372	17.6080402	12.4281407	14.7386935	7.21002055	14.0221106	9.80100503	8.72864322	4.56281407	-2.6100503
E	0.2556	0.741	15.0060302	10.8964824	12.11155779	8.26834171	6.50552764	8.06733668	10.1145729	8.16984925	7.68844221	5.933668342	-2.5839236
F	0.0678	0.247	18.681407	11.3577889	12.03001508	16.2452161	11.5819095	9.81105528	8.17688442	13.8763819	4.72562814	3.062311598	-2.59497487
G	0.018	0.082	20.9306533	9.65427136	11.132466332	8.12361809	14.2371859	7.61306533	5.4080402	7.99095477	10.8653266	9.254271357	-2.70050251
H	0	0	11.9085427	9.74271357	9.339698492	6.20201005	8.82211055	12.7788945	7.94472362	8.35075377	5.85025126	5.755778894	-2.6050251



	Concentration (ng/ml)	Absorbance	Absorbance (without noise)
A	60	2.5372	2.3933
B	20	2.2075	2.0636
C	6.667	1.2976	1.537
D	2.222	0.692	0.5481
E	0.741	0.3995	0.2556
F	0.247	0.2117	0.0678
G	0.082	0.1619	0.018
H	0	0.1439	0

Appendix D14. Manufacturer's instructions for Human sICAM-1 ELISA kit.



Millipore.

www.sigmaaldrich.com

Certificate of Analysis / Protocol

3050 Spruce Street, Saint Louis, MO 63103 USA
Tel: (800) 521-8956 (314) 771-5765 Fax: (800) 325-5052 (314)
771-5757

Product Name Human sICAM1 ELISA Kit
for serum, plasma, and cell culture supernatants
Product Number RAB0219
Lot Number 0528F0185

Storage Store the kit at -20°C. It remains active for up to 1 year. Avoid repeated freeze-thaw cycles. The reconstituted standard should be stored at -20°C or -70°C (-70°C is recommended). Opened microplate strips or reagents may be stored for up to 1 month at 2-8°C. Return unused wells to the pouch containing desiccant pack and reseal along entire edge.

- Components**
1. Human sICAM-1 Antibody-coated ELISA Plate (Item A) - RAB0219A-1EA-KC: 96 wells (12 strips x 8 wells) coated with anti-Human sICAM-1.
 2. 20x Wash Buffer (Item B) - RABWASH4
 3. Lyophilized Human sICAM-1 Protein Standard (Item C) - RAB0219C-1VL-KC
 4. Biotinylated Human sICAM-1 Detection Antibody (Item F) - RAB0219F-1VL-KC
 5. HRP-Streptavidin (Item G) - RABHRP5
 6. ELISA Colorimetric TMB Reagent (HRP Substrate, Item H) - RABTMB3
 7. ELISA Stop Solution (Item I) - RABSTOP3
 8. ELISA 1x Assay/Sample Diluent Buffer A (Item D1) - RABELADA-30ML
 9. ELISA 5x Assay/Sample Diluent Buffer B (Item E1) - RABELADB-15ML

Assay/Sample Diluent Buffer dilution (Preparation, Step 2)

Assay/Sample Diluent Buffer B (Item E1) should be diluted 5-fold with deionized or distilled water before use.

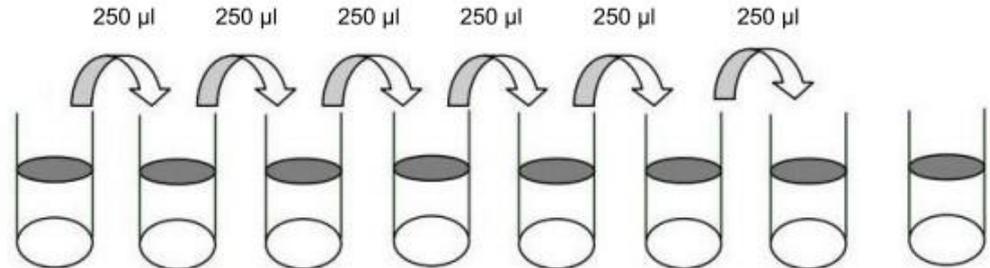
Sample Dilution (Preparation, Step 3)

Assay/Sample Diluent Buffer A (Item D1) should be used for dilution of serum and plasma samples. 1x Assay/Sample Diluent Buffer B (Item E1) should be used for dilution of cell culture supernatant samples. The suggested dilution for normal serum/plasma is 5 - 200 fold.

* Please note that the levels of sICAM-1 may vary between different samples. Optimal dilution factors for each sample must be determined by the investigator.

**Preparation of Standard
(Preparation, Step 4)**

Briefly spin a vial of Item C. Add 750 µl Assay Diluent A (for serum/plasma samples) or 1X Assay Diluent B (for cell culture medium) into Item C vial to prepare a 20 ng/ml standard. Dissolve the powder thoroughly by a gentle mix. Pipette 250 µl Assay Diluent A or 1X Assay Diluent B into each tube. Use the 20 ng/ml standard solution to produce a dilution series (Figure 1). Mix each tube thoroughly before the next transfer. Assay Diluent A or 1X Assay Diluent B serves as the zero standard (0 ng/ml). The 20 ng/ml standard in Assay Diluent B may be saturated; we recommend 10 ng/ml to serve as starting point (the highest standard point) when using Assay Diluent B.



	Std1	Std2	Std3	Std4	Std5	Std6	Std7		Zero Standard
Diluent volume	Item C + 750 µl	250 µl	250 µl	250 µl	250 µl	250 µl	250 µl		250 µl
Conc.	20 ng/ml	10 ng/ml	5 ng/ml	2.5 ng/ml	1.25 ng/ml	0.625 ng/ml	0.313 ng/ml		0 ng/ml

**Preparation of Biotinylated Detection Antibody
(Preparation, Step 6)**

Briefly spin the Detection Antibody vial (Item F) before use. Add 100 µl of 1x Diluent Buffer B (Item E1) into the vial to prepare a detection antibody concentrate. Pipette up and down to mix gently (the concentrate can be stored at 4°C for 5 days). The detection antibody concentrate should be diluted 80-fold with 1x Diluent Buffer B (Item E1) and used in Procedure, step 4.

**Dilution of HRP-Streptavidin Concentrate
(Preparation, Step 7)**

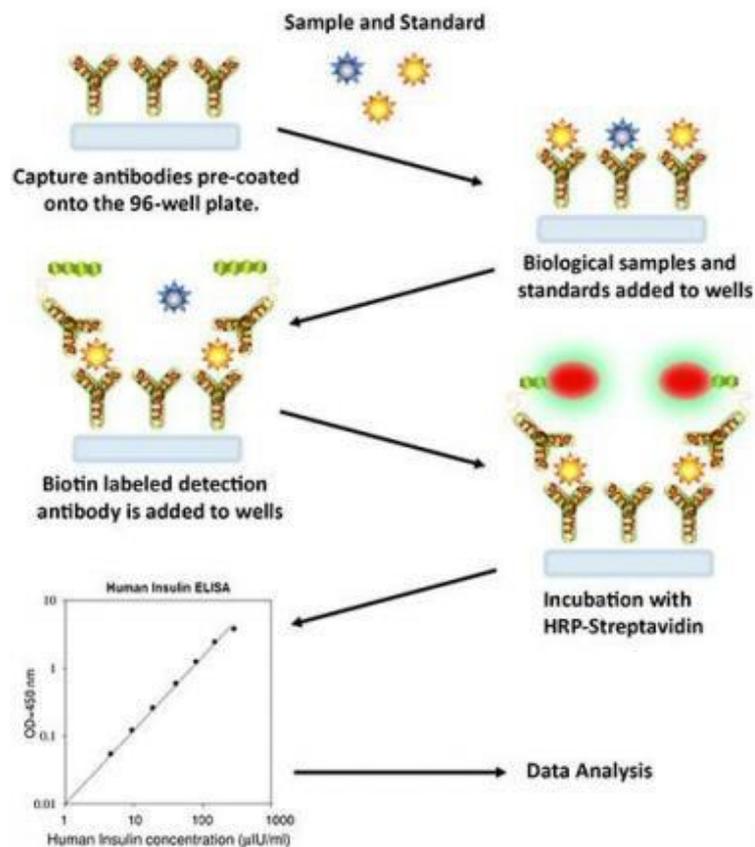
Briefly spin the HRP-Streptavidin concentrate vial (Item G) and pipette up and down to mix gently before use, as precipitates may form during storage. HRP-Streptavidin concentrate should be diluted 340-fold with 1x Diluent Buffer B (Item E1).

For example: Briefly spin the vial (Item G) and pipette up and down to mix gently. Add 25 µl of HRP-Streptavidin concentrate into a tube with 8.5 ml 1X Assay Diluent B to prepare a 340-fold diluted HRP-Streptavidin solution (don't store the diluted solution for next day use). Mix well.

Sandwich Assay Procedure

1. Bring all reagents and samples to room temperature (18 - 25 °C) before use. It is recommended that all standards and samples be run at least in duplicate.
2. Add 100 µl of each standard and sample into appropriate wells. Cover wells and incubate for 2.5 hours at room temperature or overnight at 4 °C with gentle shaking.
3. Discard the solution and wash 4 times with 1X Wash Solution. Wash by filling each well with Wash Buffer (300 µl) using a multi-channel Pipette or autowasher. Complete removal of liquid at each step is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against clean paper towels.
4. Add 100 µl of 1x prepared Detection Antibody to each well. Cover wells and incubate for 1 hour at room temperature with gentle shaking.
5. Discard the solution. Repeat the wash procedure as in step 3.
6. Add 100 µl of prepared Streptavidin solution to each well. Cover wells and incubate for 45 minutes at room temperature with gentle shaking.
7. Discard the solution. Repeat the wash as in step 3.
8. Add 100 µl of TMB One-Step Substrate Reagent (Item H) to each well. Cover wells and incubate for 30 minutes at room temperature in the dark with gentle shaking.
9. Add 50 µl of Stop Solution (Item I) to each well. Read absorbance at 450 nm immediately.

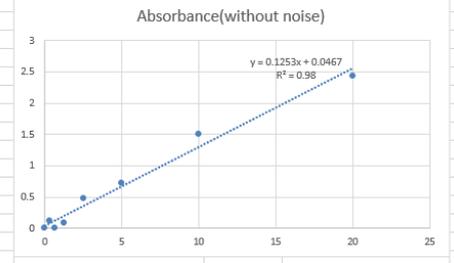
Fig 2: Example of the Sandwich ELISA process



A	2.9259	1.2468	1.0395	1.5507	1.2566	1.3307	1.5412	1.3017	1.0851	1.1201	1.3959	1.8128
B	2.0042	1.4895	1.2618	1.6771	1.1339	1.4964	1.4614	1.4633	1.2148	1.3789	1.3417	1.7042
C	1.2224	2.8361	1.429	0.9544	1.3237	1.1606	1.0782	1.2035	1.548	1.722	1.1644	1.2343
D	0.9768	1.6073	1.2878	1.5484	1.5596	1.2795	1.224	0.9273	1.304	1.0899	1.4343	1.2384
E	0.5732	1.5393	0.9441	1.7451	1.2987	1.6216	1.2762	1.2503	0.9945	1.3482	1.513	1.3134
F	0.5068	1.4537	0.9604	1.6123	1.656	1.2876	1.2413	1.0878	1.3414	1.2755	1.3908	1.1494
G	0.6071	1.9042	1.8845	1.4825	1.3714	1.2065	1.8697	1.0333	1.3234	1.2363	2.0498	1.6599
H	0.4959	1.6822	1.82	1.3803	1.5236	1.391	1.6018	1.1551	1.264	1.1155	0.9601	2.0227

20	2.9259	2.43
10	2.0042	1.5083
5	1.2224	0.7265
2.5	0.9768	0.4809
1.25	0.5732	0.0773
0.625	0.5068	0.0109
0.313	0.6071	0.1112
0	0.4959	0

End Time 2019-11-20 17:09:53												
	1	2	3	4	5	6	7	8	9	10	11	12
A	2.43	0.7509	0.5436	1.0548	0.7607	0.8348	1.0453	0.8058	0.5892	0.6242	0.9	1.3169
B	1.5083	0.9936	0.7659	1.1812	0.638	1.0005	0.9655	0.9674	0.7189	0.883	0.8458	1.2083
C	0.7265	2.3402	0.9331	0.4585	0.8278	0.6647	0.5823	0.7076	1.0521	1.2261	0.6685	0.7384
D	0.4809	1.1114	0.7919	1.0525	1.0637	0.7836	0.7281	0.4314	0.8081	0.594	0.9384	0.7425
E	0.0773	1.0434	0.4482	1.2492	0.8028	1.1257	0.7281	0.7544	0.4986	0.8523	1.0171	0.8175
F	0.0109	0.9578	0.4645	1.1164	1.1601	0.7917	0.7454	0.5919	0.8455	0.7796	0.8949	0.6535
G	0.1112	1.4083	1.3886	0.9866	0.8755	0.7106	1.3738	0.5374	0.8275	0.7404	1.5539	1.164
H	0	1.1863	1.3241	0.8844	1.0277	0.8951	1.1059	0.6592	0.7681	0.6196	0.4642	1.5268
	1	2	3	4	5	6	7	8	9	10	11	12
A	20	5.62011	3.96568	8.04549	5.69832	6.2897	7.96967	6.05826	4.32961	4.60894	6.81006	10.1373
B	10	7.55706	5.73982	9.05427	4.71907	7.61213	7.3328	7.34796	5.36472	6.67438	6.37749	9.27055
C	5	14.9497	7.07422	3.28651	6.23384	4.93216	4.27454	5.27454	8.02394	9.41261	4.96249	5.52035
D	2.5	8.49721	2.59298	8.02713	8.11652	5.88109	5.43815	3.07023	6.07662	4.36792	7.11652	5.55307
E	1.25	7.95451	3.20431	9.59697	6.03432	8.61133	5.43815	5.64804	3.60654	6.42937	7.74461	6.15164
F	0.625	7.27135	3.3344	8.53711	8.88587	5.94573	5.57622	4.35116	6.3751	5.84916	6.76935	4.84278
G	0.313	10.8667	10.7095	7.5012	6.61453	5.29848	10.5914	3.9162	6.23144	5.53631	12.0287	8.917
H	0	9.09497	10.1947	6.68555	7.82921	6.77095	8.45331	4.88827	5.75738	4.57223	3.332	11.8125
	1	2	3	4	5	6	7	8	9	10	11	12
A	20	281.006	198.284	402.275	284.916	314.485	398.484	302.913	216.48	230.447	340.503	506.864
B	10	377.853	286.991	452.713	235.954	380.607	366.64	367.398	268.236	333.719	318.875	463.528
C	5	747.486	353.711	164.326	311.692	246.608	213.727	263.727	401.197	470.63	248.125	276.018
D	2.5	424.86	129.649	401.357	405.826	294.054	271.907	153.512	303.831	218.396	355.826	277.654
E	1.25	397.725	160.215	479.848	301.716	430.567	271.907	282.402	180.327	321.468	387.231	307.582
F	0.625	363.567	166.72	426.856	444.294	297.287	278.811	217.558	318.755	292.458	338.468	242.139
G	0.313	543.336	535.475	375.06	330.726	264.924	529.569	195.81	311.572	276.816	601.437	445.85
H	0	454.749	509.737	334.278	391.46	338.547	422.666	244.413	287.869	228.611	166.6	590.623



$x = (y - 0.0467) / 0.1253$

Appendix D15. Manufacturer's instructions for Human E-selectin ELISA kit.

SIGMA-ALDRICH™

sigma-aldrich.com

Certificate of Analysis

3050 Spruce Street, Saint Louis, MO 63103 USA
Tel: (800) 521-8956 (314) 771-5765 Fax: (800) 325-5052 (314) 771-5757

Product Name Human E-Selectin ELISA Kit
for serum, plasma, and cell culture supernatants
Product Number RAB0422
Lot Number 0410F0118

Storage

Store the kit at -20°C. It remains active for up to 1 year. Avoid repeated freeze-thaw cycles. The reconstituted standard should be stored at -20°C or -70°C (-70°C is recommended). Opened microplate strips or reagents may be stored for up to 1 month at 2-8°C. Return unused wells to the pouch containing desiccant pack and reseal along entire edge.

Components

1. Human E-Selectin Antibody-coated ELISA Plate (Item A) - RAB0422A-EA: 96 wells (12 strips x 8 wells) coated with anti-Human E-Selectin.
2. 20x Wash Buffer (Item B) - RABWASH4
3. Lyophilized Human E-Selectin Protein Standard (Item C) - RAB0422C-1VL
4. Biotinylated Human E-Selectin Detection Antibody (Item F) - RAB0422D-1VL
5. HRP-Streptavidin (Item G) - RABHRP5
6. ELISA Colorimetric TMB Reagent (HRP Substrate, Item H) - RABTMB3
7. ELISA Stop Solution (Item I) - RABSTOP3
8. ELISA 1x Assay/Sample Diluent Buffer A (Item D1) - RABELADA-30ML
9. ELISA 5x Assay/Sample Diluent Buffer B (Item E1) - RABELADB-15ML

**Assay/Sample Diluent Buffer dilution
(Preparation, Step 2)**

Assay/Sample Diluent Buffer B (Item E1) should be diluted 5-fold with deionized or distilled water before use.

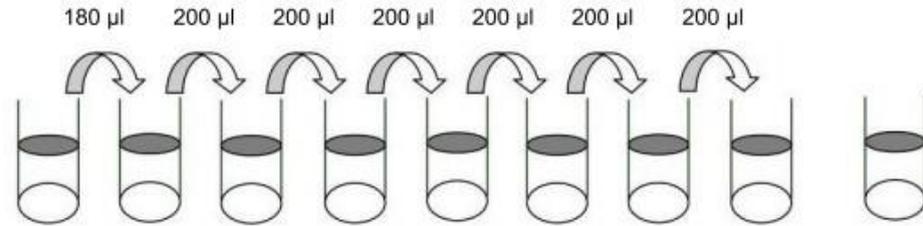
**Sample Dilution
(Preparation, Step 3)**

Assay/Sample Diluent Buffer A (Item D1) should be used for dilution of serum and plasma samples. 1x Assay/Sample Diluent Buffer B (Item E1) should be used for dilution of cell culture supernatant samples. The suggested dilution for normal serum/plasma is 50 - 200 fold.

* Please note that the levels of E-Selectin may vary between different samples. Optimal dilution factors for each sample must be determined by the investigator.

**Preparation of Standard
(Preparation, Step 4)**

Briefly spin a vial of Item C. Add 400 µl Assay Diluent A (for serum/plasma samples) or 1X Assay Diluent B (Assay Diluent B should be diluted 5-fold with deionized or distilled water, for cell culture medium) into Item C vial to prepare a 50 ng/ml standard. Dissolve the powder thoroughly by a gentle mix. Add 180 µl E-Selectin standard from the vial of Item C, into a tube with 320 µl Assay Diluent A or 1X Assay Diluent B to prepare a 18,000 pg/ml stock standard solution. Pipette 400 µl Assay Diluent A or 1X Assay Diluent B into each tube. Use the stock standard solution to produce a dilution series (Figure 1). Mix each tube thoroughly before the next transfer. Assay Diluent A or 1X Assay Diluent B serves as the zero standard (0 pg/ml). 18,000 pg/ml in Assay Diluent A (for serum/plasma samples) may be saturated, you can start from 6,000 pg/ml as the highest standard point.



		Std1	Std2	Std3	Std4	Std5	Std6	Std7		Zero Standard
Diluent volume	Item C+ 400 µl	320 µl	400 µl	400 µl	400 µl	400 µl	400 µl	400 µl		400 µl
Conc.	50 ng/ml	18000 pg/ml	6000 pg/ml	2000 pg/ml	666.7 pg/ml	222.2 pg/ml	74.07 pg/ml	24.69 pg/ml		0 pg/ml

**Preparation of Biotinylated
Detection Antibody
(Preparation, Step 6)**

Briefly spin the Detection Antibody vial (Item F) before use. Add 100 µl of 1x Diluent Buffer B (Item E1) into the vial to prepare a detection antibody concentrate. Pipette up and down to mix gently (the concentrate can be stored at 4°C for 5 days). The detection antibody concentrate should be diluted 80-fold with 1x Diluent Buffer B (Item E1) and used in Procedure, step 4.

**Dilution of HRP-Streptavidin
Concentrate
(Preparation, Step 7)**

Briefly spin the HRP-Streptavidin concentrate vial (Item G) and pipette up and down to mix gently before use, as precipitates may form during storage. HRP-Streptavidin concentrate should be diluted 900-fold with 1x Diluent Buffer B (Item E1).

For example: Briefly spin the vial (Item G) and pipette up and down to mix gently. Add 15 µl of HRP-Streptavidin concentrate into a tube with 13.5 ml 1X Assay Diluent B to prepare a final 900 fold diluted HRP-Streptavidin solution (don't store the diluted solution for next day use). Mix well.

	1	2	3	4	5	6	7	8	9	10	11	12	Concentration (ng/ml)	Absorbance	Absorbance (without noise)
A	0.6564	0.0652	0.1144	0.0805	0.0729	0.0845	0.0411	0.1011	0.0618	0.1087	0.1022	0.1319	18000	0.6564	0.6091
B	0.3967	0.1329	0.1252	0.1038	0.0971	0.1177	0.1371	0.1123	0.0989	0.14	0.0847	0.1096	6000	0.3967	0.3494
C	0.1771	0.1457	0.0839	0.0879	0.0877	0.1653	0.1194	0.0855	0.0878	0.1602	0.0826	0.0944	2000	0.1771	0.1298
D	0.0862	0.085	0.0697	0.0637	0.08	0.0802	0.0786	0.0767	0.0844	0.0478	0.1057	0.0767	666.7	0.0862	0.0389
E	0.0591	0.1162	0.0733	0.0714	0.0854	0.1177	0.0792	0.0791	0.1016	0.1318	0.0887	0.071	222.2	0.0591	0.0118
F	0.0553	0.114	0.0834	0.0793	0.139	0.1025	0.0936	0.0805	0.1107	0.1179	0.133	0.0966	74.07	0.0553	0.008
G	0.0525	0.0952	0.1095	0.0652	0.0919	0.1363	0.1223	0.0803	0.1099	0.1021	0.2075	0.0884	24.69	0.0525	0.0052
H	0.0473	0.1354	0.1285	0.1229	0.1657	0.1335	0.1159	0.1239	0.148	0.0928	0.0985	0.1381	0	0.0473	0

	1	2	3	4	5	6	7	8	9	10	11	12
A	0.6091	0.0179	0.0671	0.0332	0.0256	0.0372	-0.0062	0.0538	0.0145	0.0614	0.0549	0.0846
B	0.3494	0.0856	0.0779	0.0565	0.0498	0.0704	0.0898	0.065	0.0516	0.0927	0.0374	0.0623
C	0.1298	0.0984	0.0366	0.0406	0.0404	0.118	0.0721	0.0382	0.0405	0.1129	0.0353	0.0471
D	0.0389	0.0377	0.0224	0.0164	0.0327	0.0329	0.0313	0.0294	0.0371	0.0005	0.0584	0.0294
E	0.0118	0.0689	0.026	0.0241	0.0381	0.0704	0.0319	0.0318	0.0543	0.0845	0.0414	0.0237
F	0.008	0.0667	0.0361	0.032	0.0917	0.0552	0.0463	0.0332	0.0634	0.0706	0.0857	0.0493
G	0.0052	0.0479	0.0622	0.0179	0.0446	0.089	0.075	0.033	0.0626	0.0548	0.1602	0.0411
H	0	0.0881	0.0812	0.0756	0.1184	0.0862	0.0686	0.0766	0.1007	0.0455	0.0512	0.0908

	1	2	3	4	5	6	7	8	9	10	11	12
A	18000	313.333	1326.67	196.667	56.6667	330	0	883.333	426.667	1136.67	920	1910
B	6000	1943.33	1686.67	973.333	750	1436.67	2083.33	1256.67	810	2180	336.667	1166.67
C	2000	2370	310	443.333	436.667	3023.33	1493.33	363.333	440	2853.33	266.667	660
D	666.7	346.667	163.333	363.333	180	186.667	133.333	70	326.667	893.333	1036.67	70
E	222.2	1386.67	43.3333	106.667	360	1436.67	153.333	150	900	1906.67	470	120
F	74.07	1313.33	293.333	156.667	2146.67	930	633.333	196.667	1203.33	1443.33	1946.67	733.333
G	24.69	686.667	1163.33	313.333	576.667	2056.67	1590	190	1176.67	916.667	4430	460
H	0	2026.67	1796.67	1610	3036.67	1963.33	1376.67	1643.33	2446.67	606.667	796.667	2116.67

	1	2	3	4	5	6	7	8	9	10	11	12
A	18000	19896.7	84243.3	12488.3	3598.33	20955	910	56091.7	27093.3	72178.3	58420	121285
B	6000	123402	107103	61806.7	47625	91228.3	132292	79798.3	51435	138430	21378.3	74083.3
C	2000	150495	19685	28151.7	27728.3	191982	94826.7	23071.7	27940	181187	16933.3	41910
D	666.7	22013.3	10371.7	23071.7	11430	11853.3	8466.67	4445	20743.3	56726.7	65828.3	4445
E	222.2	88053.3	2751.67	6773.33	22860	91228.3	9736.67	9525	57150	121073	29845	7620
F	74.07	83396.7	18626.7	9948.33	136313	59055	40216.7	12488.3	76411.7	91651.7	123613	46566.7
G	24.69	43603.3	73871.7	19896.7	36618.3	130598	100965	12065	74718.3	58208.3	281305	29210
H	0	128693	114088	102235	192828	124672	87418.3	104352	155363	38523.3	50588.3	134408

	P1V2	P9V2	P17V2	P25V2	P1V4	P9V4	P17V4	P25V4	P1V1	P21V1	P5V3
A											
B											
C											
D											
E											
F											
G											
H											

Concentration (ng/ml)	Absorbance	Absorbance (without noise)
18000	0.6564	0.6091
6000	0.3967	0.3494
2000	0.1771	0.1298
666.7	0.0862	0.0389
222.2	0.0591	0.0118
74.07	0.0553	0.008
24.69	0.0525	0.0052
0	0.0473	0

Absorbance (without noise) $y = 3E-05x + 0.0273$
 $r^2 = 0.947$

3e-05 value = 0.00003
 $x = (y - 0.0273) / 0.00003$

Appendix D16. Manufacturer's instructions for Human Syndecan-1 ELISA kit.



www.sigmaldrich.com

Certificate of Analysis / Protocol 3050 Spruce Street, Saint Louis, MO 63103 USA
Tel: (800) 521-8956 (314) 771-5765 Fax: (800) 325-5052 (314)
771-5757

Product Name Human SDC1 / Syndecan-1 ELISA Kit
for serum, plasma, and cell culture supernatants
Product Number RAB0736
Lot Number 0612F0035

Storage Store the kit at -20°C. It remains active for up to 1 year. Avoid repeated freeze-thaw cycles. The reconstituted standard should be stored at -20°C or -70°C (-70°C is recommended). Opened microplate strips or reagents may be stored for up to 1 month at 2-8°C. Return unused wells to the pouch containing desiccant pack and reseal along entire edge.

- Components**
1. Human Syndecan 1 Antibody-coated ELISA Plate (Item A) - RAB0736A-1EA-KC: 96 wells (12 strips x 8 wells) coated with anti-Human Syndecan 1.
 2. 20x Wash Buffer (Item B) - RABWASH4
 3. Lyophilized Human Syndecan 1 Protein Standard (Item C) - RAB0736C-1VL-KC
 4. Biotinylated Human Syndecan 1 Detection Antibody (Item F) - RAB0736F-1VL-KC
 5. HRP-Streptavidin (Item G) - RABHRP5
 6. ELISA Colorimetric TMB Reagent (HRP Substrate, Item H) - RABTMB3
 7. ELISA Stop Solution (Item I) - RABSTOP3
 8. ELISA 5x Assay/Sample Diluent Buffer (Item E2) - RABELADE-15ML

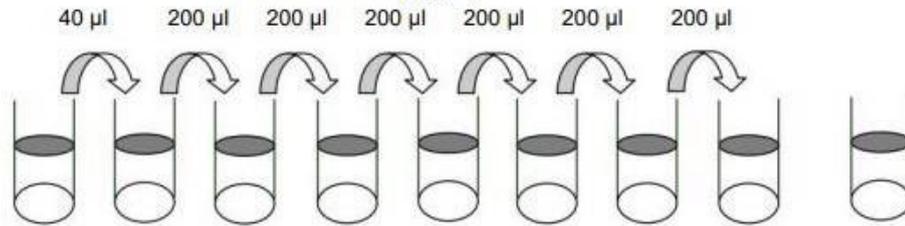
Assay/Sample Diluent Buffer dilution (Preparation, Step 2) Assay/Sample Diluent Buffer (Item E2) should be diluted 5-fold with deionized or distilled water before use.

Sample Dilution (Preparation, Step 3) 1x Assay/Sample Diluent Buffer (Item E2) should be used for dilution of serum, plasma, and cell culture supernatant samples. The suggested dilution for normal serum/plasma is 8 fold.

* Please note that the levels of Syndecan 1 may vary between different samples. Optimal dilution factors for each sample must be determined by the investigator.

**Preparation of Standard
(Preparation, Step 4)**

Briefly spin a vial of Item C. Add 400 µl 1X Assay Diluent (Item E2; Assay Diluent should be diluted 5-fold with deionized or distilled water before use) into Item C vial to prepare a 50 ng/ml standard solution. Dissolve the powder thoroughly by a gentle mix. Add 40 µl of the Syndecan-1 standard solution from the vial of Item C, into a tube with 460 µl 1X Assay Diluent to prepare a 4,000 pg/ml standard solution. Pipette 300 µl 1X Assay Diluent into each tube. Use the 4,000 pg/ml standard solution to produce a dilution series (Figure 1). Mix each tube thoroughly before the next transfer. 1X Assay Diluent serves as the zero standard (0 pg/ml).



		Std1	Std2	Std3	Std4	Std5	Std6	Std7	Zero Standard
Diluent volume	Item C+ 400 µl	460 µl	300 µl	300 µl	300 µl	300 µl	300 µl	300 µl	300 µl
Conc.	50 ng/ml	4000 pg/ml	1600 pg/ml	640 pg/ml	256 pg/ml	102.4 pg/ml	40.96 pg/ml	16.38 pg/ml	0 pg/ml

**Preparation of Biotinylated
Detection Antibody
(Preparation, Step 6)**

Briefly spin the Detection Antibody vial (Item F) before use. Add 100 µl of 1x Diluent Buffer (Item E2) into the vial to prepare a detection antibody concentrate. Pipette up and down to mix gently (the concentrate can be stored at 4°C for 5 days). The detection antibody concentrate should be diluted 80-fold with 1x Diluent Buffer (Item E2) and used in Procedure, step 4.

**Dilution of HRP-Streptavidin
Concentrate
(Preparation, Step 7)**

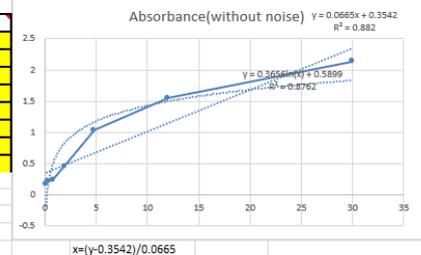
Briefly spin the HRP-Streptavidin concentrate vial (Item G) and pipette up and down to mix gently before use, as precipitates may form during storage. HRP-Streptavidin concentrate should be diluted 200-fold with 1x Diluent Buffer (Item E2).

For example: Briefly spin the vial (Item G) and pipette up and down to mix gently. Add 60 µl of HRP-Streptavidin concentrate into a tube with 12 ml 1X Assay Diluent to prepare a 200-fold diluted HRP-Streptavidin solution (don't store the diluted solution for next day use). Mix well.

	1	2	3	4	5	6	7	8	9	10	11	12
A	2.2305	0.2718	0.3353	0.3928	0.3777	0.5978	0.3174	0.3778	0.3707	0.3561	0.1747	0.4052
B	1.6422	0.4057	0.3968	0.4088	0.3912	0.4392	0.3951	0.4164	0.4791	0.3784	0.6955	0.3737
C	1.1221	0.834	0.4479	0.5294	0.6312	0.4516	0.4916	0.4054	0.5235	0.395	0.6192	0.438
D	0.5389	1.3383	0.6883	1.2222	0.4705	0.7086	0.6604	0.8126	0.5314	0.3199	1.2948	0.7657
E	0.3295	1.0767	0.6468	0.7639	1.5506	0.8396	0.6904	0.2987	1.7153	0.4651	0.4547	0.4197
F	0.3099	0.951	0.7468	0.4681	0.6516	0.6644	0.4828	0.5694	0.5987	0.459	0.4364	0.5937
G	0.2564	0.3016	0.2238	0.8103	0.5533	0.4618	0.3705	0.5113	0.6745	0.278	0.1458	0.5234
H	0.0901	0.1379	0.1116	0.7837	0.1096	0.334	0.2489	0.8425	0.2258	0.5669	0.1883	0.5095

	Concentration (ng/ml)	Absorbance	Absorbance(without noise)
A	30	2.2305	2.1404
B	12	1.6422	1.5521
C	4.8	1.1221	1.032
D	1.92	0.5389	0.4488
E	0.768	0.3295	0.2394
F	0.307	0.3099	0.2198
G	0.123	0.2564	0.1663
H	0	0.0901	0

	Absorbance	2	3	4	5	6	7	8	9	10	11	12	100
A	2.1404	0.1817	0.2452	0.3027	0.2876	0.5077	0.2273	0.2877	0.2806	0.266	0.0846	0.3151	30
B	1.5521	0.3156	0.3067	0.3187	0.3011	0.3491	0.305	0.3263	0.389	0.2883	0.6054	0.2836	12
C	1.032	0.7439	0.3578	0.4393	0.5411	0.3615	0.4015	0.3153	0.4334	0.3049	0.5291	0.3479	4.8
D	0.4488	1.2482	0.5982	1.1321	0.3804	0.6185	0.5703	0.7225	0.4413	0.2298	1.2047	0.6756	1.92
E	0.2394	0.9866	0.5567	0.6738	1.4605	0.7495	0.6003	0.2086	1.6252	0.375	0.3646	0.3296	0.768
F	0.2198	0.8609	0.6567	0.378	0.5615	0.5743	0.3927	0.4793	0.5086	0.3689	0.3463	0.5036	0.307
G	0.1663	0.2115	0.1337	0.7202	0.4632	0.3717	0.2804	0.4212	0.5844	0.1879	0.0557	0.4333	0.123
H	0	0.0478	0.0215	0.6936	0.0195	0.2439	0.1588	0.7524	0.1357	0.4768	0.0982	0.4194	0



	Absorbance	2	3	4	5	6	7	8	9	10	11	12
A	30	2.593985	1.639098	0.774436	1.001504	2.308271	1.908271	1	0.971429	1.326316	4.054135	0.58797
B	12	0.580451	0.714286	0.533835	0.798496	0.076692	0.73985	0.419549	0.523308	0.990977	3.777444	1.061654
C	4.8	5.86015	0.054135	1.279699	2.810526	0.109774	0.711278	0.584962	1.190977	0.741353	2.630075	0.094737
D	1.92	13.44361	3.669173	11.69774	0.393985	3.974436	3.249624	5.538346	1.309774	1.870677	12.78947	4.833083
E	0.768	9.509774	3.045113	4.806015	16.63609	5.944361	3.700752	2.189474	19.11278	0.312782	0.156391	0.369925
F	0.307	7.619549	4.548872	0.357895	3.117293	3.309774	0.578947	1.881203	2.321805	0.221053	0.118797	2.246617
G	0.123	2.145865	3.315789	5.503759	1.639098	0.263158	1.109774	1.007519	3.461654	2.500752	4.488722	1.189474
H	0	4.607519	0.500308	5.103759	5.033083	1.658647	2.938346	5.98797	3.285714	1.843609	3.849624	0.980451

	Absorbance	2	3	4	5	6	7	8	9	10	11	12
A	30	5.18797	3.278195	1.548872	2.003008	4.616541	3.816541	2	1.942857	2.652632	8.108271	1.17594
B	12	1.160902	1.428571	1.067669	1.596992	0.153383	1.479699	0.839098	1.046617	1.981959	7.554887	1.23308
C	4.8	11.7203	0.108271	2.593988	5.621053	0.219549	1.422556	1.169925	2.381955	1.482707	5.26015	0.189474
D	1.92	26.88722	7.338346	23.39549	0.78797	7.948872	6.499248	11.07669	2.619549	3.741353	25.57895	9.666165
E	0.768	19.01955	6.090226	9.61203	33.27218	11.88872	7.401504	4.378947	38.22556	0.625564	0.312782	0.73985
F	0.307	15.2391	9.097744	0.715789	6.234586	6.619549	1.157895	3.762406	4.643609	0.442105	0.237594	4.493233
G	0.123	4.291729	6.831579	11.00752	3.278195	0.526316	2.219549	2.015038	6.923308	5.001504	8.977444	2.378947
H	0	9.215038	10.00602	10.20752	10.06617	3.317293	5.876692	11.97594	6.571429	3.687212	7.699248	1.969092

Appendix D17. G-power sample size calculation

G*Power 3.1.9.4

File Edit View Tests Calculator Help

Central and noncentral distributions Protocol of power analyses

critical t = 2.06866

Test family: **t tests**

Statistical test: **Means: Difference between two dependent means (matched pairs)**

Type of power analysis: **A priori: Compute required sample size – given α , power, and effect size**

Input Parameters

Tail(s): **Two**

Determine =>

Effect size dz: **0.6000000**

α err prob: **0.05**

Power ($1 - \beta$ err prob): **0.80**

Output Parameters

Noncentrality parameter δ : **2.9393877**

Critical t: **2.0686576**

Df: **23**

Total sample size: **24**

Actual power: **0.8036714**

X-Y plot for a range of values **Calculate**

From differences

Mean of difference: **0**

SD of difference: **1**

From group parameters

Mean group 1: **111**

Mean group 2: **105**

SD group 1: **10**

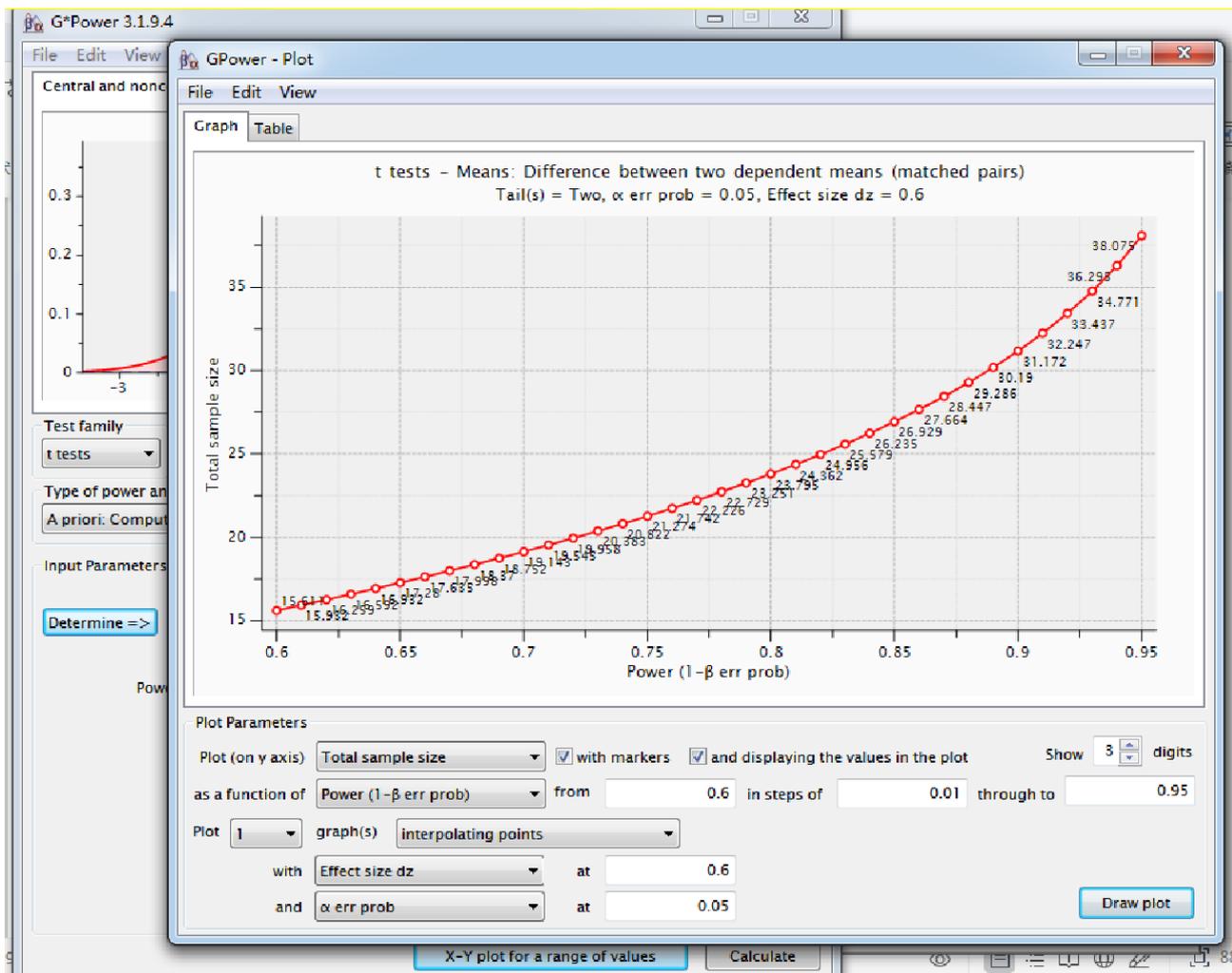
SD group 2: **10**

Correlation between groups: **0.5**

Calculate Effect size dz: **0.6**

Calculate and transfer to main window

Close



		Olive Oil Baseline			Olive Oil post			Butter Baseline			Butter Post		
		Systolic	Diastolic	MAP	Systolic	Diastolic	MAP	Systolic	Diastolic	MAP	Systolic	Diastolic	MAP
1	MM	111.0	72.0	87.0	107.0	66.0	81.0	111.0	67.0	82.0	104.0	60.0	75.0
2	FG	119.0	68.0	87.0	125.0	68.0	88.0	124.0	67.0	88.0	129.0	73.0	92.0
3	YY	134.0	88.0	103.0	134.0	89.0	103.0	118.0	71.0	86.0	123.0	76.0	91.0
4	JH	125.0	75.0	90.0	123.0	72.0	90.0	122.0	71.0	88.0	117.0	67.0	83.0
5	Adam	125.3	67.5	86.8	120.0	69.0	88.0	118.8	67.5	84.6	122.4	71.8	88.7
6	RD	134.0	80.0	95.0	115.3	68.0	83.7	138.0	90.0	104.0	136.0	77.0	94.0
7	JTB	126.0	73.0	90.0	121.0	70.0	88.0	122.6	69.9	87.0	142.0	77.4	98.9
8	SS	119.3	57.5	78.1	108.6	61.2	77.0	121.3	66.2	84.6	127.3	68.3	87.3
9	MC	134.4	80.8	98.7	127.6	68.3	88.1	148.0	101.0	117.0	126.0	84.0	98.0
10	LuP	112.0	61.0	78.0	119.0	67.0	84.0	110.1	60.7	77.1	130.1	64.4	86.3
11	NN	118.0	71.0	88.0	120.0	74.0	90.0	115.4	65.0	82.3	123.2	65.3	84.6
12	JH2	133.0	66.0	90.0	130.0	74.0	93.0	132.8	70.3	91.1	118.8	61.8	80.8
13	SC	115.7	57.7	77.0	110.8	54.4	73.2	118.0	68.0	87.0	119.7	66.4	84.2
14	IJ	133.9	68.8	90.5	139.0	81.0	101.0	127.0	77.0	93.0	121.0	76.0	93.0
15	TS	123.0	71.0	88.0	119.0	69.0	86.0	122.8	64.8	84.2	125.0	74.0	90.0
16	JJ	116.0	56.0	76.0	120.0	59.0	79.0	111.5	64.5	80.1	124.0	63.3	83.6
17	STC	113.8	64.0	80.6	114.0	56.0	79.0	115.2	59.7	78.2	120.0	64.0	84.0
18	AH	105.0	60.0	74.0	110.5	64.9	80.6	107.0	65.0	79.0	108.0	66.0	81.0
1	Dinh	117.0	76.0	90.0	130.0	81.0	96.0	119.0	74.0	88.0	131.9	83.3	99.5
2	KTC	116.0	70.0	86.0	118.0	63.0	84.0	118.9	76.0	90.3	121.0	67.0	85.0
3	LWD	122.8	79.4	92.0	104.5	75.1	84.9	115.0	76.8	89.5	122.7	67.2	85.7
4	WL	125.5	62.4	83.4	123.0	74.0	89.0	114.0	62.0	79.3	126.0	73.0	92.0
5	AW	124.8	63.9	84.2	113.2	65.8	81.9	114.0	64.0	83.0	116.5	60.8	79.4
6	HMC	113.0	68.0	85.0	113.0	71.0	86.0	115.0	70.0	87.0	114.0	70.0	85.0
7	ZJN	125.0	74.6	91.4	113.1	60.8	78.3	124.0	67.3	86.2	130.0	70.5	90.3
8	JR	116.5	64.8	82.0	105.7	63.2	77.4	113.5	66.3	82.0	120.7	73.7	89.3
9	JC	120.0	72.0	88.0	114.0	67.0	83.0	111.8	62.0	78.6	115.0	67.0	84.0
10	RHB	116.0	75.0	88.0	118.0	78.0	90.0	119.0	75.0	91.0	109.0	69.0	84.0
11	ZY	120.5	63.5	82.5	114.0	70.0	86.0	118.0	75.0	90.0	116.0	73.0	88.0
12	BRR	108.0	64.0	79.0	108.7	64.1	79.0	106.2	65.8	79.2	109.0	64.0	79.0
13	XL	114.5	63.2	80.3	110.0	59.0	80.0	116.0	71.0	86.0	119.0	69.5	86.0
14	RH	124.4	72.0	89.5	120.9	69.8	86.9	130.0	85.0	100.0	138.0	94.0	109.0

Appendix D18.

Intervention group
Filippo Berio Organic Extra Virgin Olive Oil (500ml)



Control group
Lurpak Unsalted Block Butter 250G



Appendix D19.

THREE-DAY DIETARY RECORD

PROJECT TITLE: Effect of olive oil consumption on cardiovascular biomarkers in Asians and Caucasians: A randomized, crossover, controlled interventional trial

RESEARCHER: Fan Liang

PRINCIPAL INVESTIGATOR: Dr Jose Lara Gallegos

This Food Record Belongs to: _____	
Participant Number: _____	Visit: _____
Name (print): _____	Age: _____
You should record my diet intake for these following three days:	
Day 1 date: _____	
Day 2 date: _____	
Day 3 date: _____	

**You are receiving these instructions because the research study you are participating in asks that you keep a diet record for a specified number of days. If after reading these instructions you have further questions regarding your diet record, please contact Fan Liang fan.liang@northumbria.ac.uk.*

- **It is important that this record be both *accurate* and *representative* of your normal dietary intake.** Thus it is essential that you do not alter your normal eating habits in any way while keeping this diet record and that you record as precisely as possible every single item that you consume (this includes water, vitamins, condiments, etc.). To do so, you must follow a few simple instructions (listed below). The purpose here is to correctly record and quantify your normal intake, not to judge it. If you change your eating habits in any way, then we cannot accurately analyze your typical diet. The procedure may seem somewhat cumbersome, but remember, it is **only three days**.
- **INSTRUCTIONS for RECORDING YOUR FOOD INTAKE:**
 1. **Keep a pen and paper with you at all times to record your intake including food item, quantity, and notes.** This is imperative as snacks are typically consumed unpredictably and, as a result, it is impossible to record them accurately unless your recording forms are nearby.

2. **Please be as accurate as possible in recording amounts.** Use standard measuring tools (measuring cups/spoons, food scales if available) to measure food portions consumed. If measuring tools are not available, you must estimate the portion size of what you are eating. See the portion estimate handout or other tools provided.
3. Write down **everything** you eat or drink, including water, and all vitamin / mineral supplements taken for the 3-day period. Don't forget to include snacking or food eaten while preparing a meal. Every bite counts! Ideally do not wait to record later!
4. **Only record the portion of food you actually consume.** If you do not eat the entire item (for instance a portion of an apparently delicious hastily prepared casserole of leftovers that turned out to be not so delicious), re-measure what is left and record the difference. Record combination foods separately (e.g., hot dog, bun, and condiments) and include brand names of food items (list contents of homemade items) whenever possible. For packaged items, use labels to determine quantities.
5. **Record three days that are representative of your normal intake.** Track your food and beverage intake for a three-day period (two weekdays [Monday through Friday] and one weekend day [Saturday or Sunday]). These three days **DO NOT** need to be consecutive.
6. Remember includes **preparation method** or other details that may help describe the food, for example: indicate whether a chicken breast is baked, grilled, breaded, fried, etc. or whether vegetables are raw, steamed, "Southern style", sautéed, fried, etc. List these on a separate line. Also, do not forget to record all your beverages consumed with meals and also between meals.

- **Hunger/Fullness Rating: Use scale 1-10 by how you feel BEFORE AND AFTER you eat.**

1 ----- 2----- 3 ----- 4 ----- 5 ----- 6 ----- 7 ----- 8 ----- 9 ----- 10

starving stomach grumble Neither hungry/full pleasantly full painfully full

Example: If you feel your stomach grumble and you decide to eat, **record a 3 for hunger**. If you eat until you feel pleasantly full **record a 7 for satiety**. Recording this information can help you identify external or emotional cues to eat.

- **FOOD/BEVERAGE RECORDING INSTRUCTIONS:**

Record all food and beverages consumed during a 24 hour period. Provide the following:

- **Type of Food Eaten:** e.g. chicken noodle soup
- **Brand Name:** e.g. Campbell's, Lipton, Weight Watchers, Cheerios, Lean Cuisine, Yoplait, Heinz...
- **Restaurant Name:** McDonalds, Olive Garden, Pizza Hut, Jimmy Johns...
- **Preparation Techniques:** Grilled, boiled, fried, baked, roasted, steamed, microwave.

Food or Beverage Characteristics:

- **Colour:** e.g. green vs. yellow beans; white vs. whole wheat bread
- **Fat Content:** % fat (e.g. skim, 1%, 2% or homo milk), leanness of meat (e.g. extra lean ground beef), fat claims (e.g. "light", "low-fat"), was skin removed from poultry?
- **Freshness:** e.g. fresh, frozen, canned, or dried?
- **Other Details:** e.g. 25% reduced sodium, "diet" products, etc.
- **Time of Day:** you ate or drank

Example:

When you record an item that consists of a combination of foods, please break it down into individual components:

i.e. Turkey Sandwich

- ☞ 2 slices whole wheat bread
- ☞ 2 oz deli turkey breast, 95% lean
- ☞ 1 slice Kraft Fat Free American cheese
- ☞ 2 tsp Miracle Whip

2. Please MEASURE and describe the amount of food eaten as best as possible. Diet records are only reliable with accurate measurements.

- **Always estimate portion sizes of food after cooking.**
- **Use household measures to specify serving sizes.**
 - 1 cup = 284 milliliters (mL);
 - 1 pound = 453.6 grams (g);
 - 1 ounce (oz.) =28.35 g
 - 1 fluid ounce (fl oz.) = 28.41 g;
 - 1 gram (g) = 0.0353 oz;
 - 1 kilogram (kg) = 2.20516lb;
 - 1 pint = 568.3 mL
 - 1 litre = 1.76 pints
 - 284 mL = 9.6 fluid ounces;
 - 1 tablespoon (tbsp) = about 15 mL = ½ fl oz.
 - 1 dessertspoonful = about 10 mL = 1/4 fl oz.
 - 1 teaspoon (tsp) = about 5 mL = 1/8 fl oz.
 - 1 teacupful of solids = about 4 UK ounces = 1/4 pound(lb.) = 112 g

1/2 cup	64 g	2.25 oz
2/3 cup	85 g	3 oz
3/4 cup	96 g	3.38 oz
1 cup	128 g	4.5 oz

UK Fluid Ounces (A British cooking measurement): mL = UK fl oz / 0.035195

Please check this website of UK Fluid Ounces to Milliliters:

<https://www.metric-conversions.org/volume/uk-ounces-to-milliliters.htm>

Please check this website of UK Fluid Ounces to USA Fluid Ounces:

<https://www.metric-conversions.org/volume/uk-ounces-to-us-ounces.htm>

- **Measuring cups (examples):** Put cooked pasta or rice into a measuring cup to record the correct amount before placing it on your plate. Measure your cereal out before pouring into a bowl, and don't forget to measure your milk as well!
- **Teaspoons/tablespoons (examples):** Measure out butter, margarine, mayonnaise, salad dressings, ketchup, mustard, ground flaxseed, sugar, milk/cream, and other condiments, seasonings, and toppings before adding to your food or beverages.
- **Count the number of food items if practical:** e.g.: 20 grapes, 15 baby carrots, 8 medium-sized shrimp, etc.
- **Fluids:** Record amounts in milliliters (mL), or in grams (g).

- Use food labels to estimate quantities:** Food labels can help you estimate the quantity of food eaten based on weight or volume. **For example:** write down a 355mL can of pop, 1/2 of a 60g can of tuna, a 37g granola bar, etc.
- Use your hand to estimate portion sizes quickly:**
Whole Thumb = 1 Tablespoon; Tip of your Thumb = 1 Teaspoon; Palm of Your Hand = 3 oz;
A Fist = 1 cup



- Record if anything was ADDED when preparing the food**, such as oil (list specific kind), sauce, butter, margarine, or other condiments or seasonings.
- For COMBINATION DISHES** such as lasagna, casseroles, chili, soups, or stews include a **description of the main ingredients**. E.g. Lasagna: lean ground beef (1/4 cup per piece), mozzarella cheese (1 oz per piece), cottage cheese (1 oz per piece), 1/2 cup tomato sauce, 2 noodles, 1/4 cup spinach.
- Include SNACK FOODS eaten.** Don't forget to include candy, chips, cookies, popcorn, ice cream, and beverages such as soft drinks, juice, coffee, or tea.
- Use the "notes" column to record any additional PRODUCT INFORMATION** if available (e.g. 6 crackers – 80 calories, 2.5g fat, 1g fibre, 210mg sodium).
- Don't forget to write down any ALCOHOLIC BEVERAGES consumed and how much you drank.** This includes all wine, beer, and liquor.

Food Intake Record for (name): _____
 Date: _____ Day of week: _____ Phase/Visit #: _____

For Office Use Only: Reviewed by (initials): _____ Data entered by (initials): _____
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Below is an EXAMPLE of how to keep accurate records.

Food Record									Date: 16.09.2019
Meal	Date	Time Of Day	Consumed Food & Beverage Item	**Amount /quantity eaten	*Notes	Hunger / Fullness	Description (Brand name or restaurant name)	Preparation technique (if applicable)	
Breakfast	12/23	8:30am	Regular cheerios	1 ½ cups			General Mills		
			sugar	2 tsp					
			2% milk	1 cup; 8 fl oz			Purity		
			Fresh blueberries	¾ cup					
			Orange juice	½ cup; 6 fl oz					
			Water	1 cup					
			2 pieces of toast	2 pc			4/8		
			Becel Margarine	1 T	Salt-free				
			Mayo	1 Tbsp	light		Hellman's		
Lunch	12/23	12:45pm	Turkey sub	6 in			Subway		
			including shredded lettuce	¼ cup				Shredded	
			and tomato	¼ cup				Sliced	
			on wheat bread						
			with Cheddar Cheese Slices	2 oz					
			with light mayonnaise	1 Tbsp					
			Baked Lays Orig. Potato Crisps	1 oz			Frito-Lay		

			Diet Coke	12 Fluid ounces				
			Small pizza	400 g	Pepperoni, mushroom, cheese			
			ice cream- UDF regular Heavenly Hash	1 cup				
Evening meal	12/23	7:00pm	Salmon fillet	5 oz				baked
			with Diced Raw Tomato	½ cup				
			and Diced Raw Onion	½ cup				
			and White Wine for Cooking	½ cup				
			and Ground Black Pepper	¼ tsp				
			Long Grain Brown Rice	¾ cup				Cooked
			Salad					Tossed
			Romaine Lettuce	2 cups				Shredded
			Raw Carrots	1/3 cup				Sliced
			Raw Tomatoes	½ cup				Diced
			with Diet Italian Dressing	2 Tbsp				
			White wine	5 fl oz				
			Mixed vegetables	1 c	Peas, carrots, corn			
			Chicken	6 oz		3/7		Grilled
			Baked potato	6 oz				
Snacks	12/23	3:00pm	Apple	1 medium				
			Peanut butter	1 tbsp			Peter Pan creamy	
		8:00pm	Peanut M and M's	10			Shared pkg with friend	
			Water with lemon	2 cups				

		10:00pm	Popcorn Light Natural Flavor	2 cups			Orville	Microwaved
			Green tea	1 cup				

Was this a typical day? If not, why? Usually drink more water (forgot water bottle at home)

**Note: Include ingredients & amounts of homemade items, did this food have any type of Nutrition Claim?*

***Amount/quantity eaten: Grams, mL, number/count, ounces, tablespoons [tbsp], teaspoons[tsp], cups, leaf, patty, slice.*

- Food Intake Record for (name): _____
- Date: _____ Day of week: _____ Phase/Visit #: _____

Food Record --- Day 1					Date: _____		
Meal	Date	Time Of Day	Consumed Food & Beverage Item	Amount/quantity eaten	Notes	Hunger/ Fullness	Preparation technique
Breakfast							
Lunch							

Snacks							

Was this a typical day? If not, why? _____

- Food Intake Record for (name): _____
- Date: _____ Day of week: _____ Phase/Visit #: _____

Food Record --- Day 2					Date:			
Meal	Date	Time Of Day	Consumed Food & Beverage Item	Amount/quantity eaten	Notes	Hunger/ Fullness	Description	Preparation technique
Breakfast								
Lunch								

Evening meal								
Snacks								

Was this a typical day? If not, why? _____

- Food Intake Record for (name): _____
- Date: _____ Day of week: _____ Phase/Visit #: _____

Food Record --- Day 3						Date:		
Meal	Date	Time Of Day	Consumed Food & Beverage Item	Amount/quantity eaten	Notes	Hunger/ Fullness	Description	Preparation technique
Breakfast								
Lunch								

Evening meal								
Snacks								

Was this a typical day? If not, why? _____

Participant - 3 day dietary record sample

**Downlee Systems Limited
Downlee Lodge, Bankhall Drive
Chapel-en-le-Frith
High Peak SK23 9UB**

(To replace this text with your own heading: Prepare a file using any word processor and save it in rich text format with .rtf extension. In "Microdiet" select *File* then *Select Header for Reports* click the *Open File* button and select the .rtf file. Click the *save* button. From then on the saved text will appear at the start of your reports when the Report Header box is ticked.)

HMC 3d dietary record

03/09/2019

Food List

Code	Food Name	Quantity
Grams		
Day 1		
Breakfast		
12315	Whole milk, average	250.0 g
17-171	Tea, green, infusion	1.0 cup (190.0g)
12312	Semi-skimmed milk, average	1.0 m tea (30.0g)
17083	Sugar, white	1.0 cube (5.0g)
11512	Digestive biscuits, chocolate	1.0 each (15.0g)
Lunch		
11446	White rice, easy cook, boiled	1.0 medium por (180.0g)
Dinner		
13-525	Pepper, capsicum, red, boiled in unsalted water	1.0 medium por (30.0g)
11443	Brown rice, boiled	1.0 large por (290.0g)
18-288	Pork, mince, stewed	1.0 medium por (100.0g)
13-502	Broccoli, green, raw	1.0 medium por (85.0g)
14-322	Grapes, green	10.0 each (60.0g)
Day 2		
Breakfast		
12315	Whole milk, average	280.0 g
Mid-morning snack		
17-189	Tea, infusion, average, with semi-skimmed milk	1.0 cup (190.0g)
11-807	Biscuits, digestive, half coated in chocolate	1.0 each (15.0g)
Lunch		
11-450	Pasta, plain, fresh, boiled	1.0 large por (350.0g)
18-288	Pork, mince, stewed	1.0 medium por (100.0g)
15-871	Salad, Greek	1.0 medium por (100.0g)
Dinner		

11-880	Rice, white, basmati, easy cook, boiled in unsalted water	1.0 medium por (180.0g)
18-318	Chicken, thighs, casserole, meat and skin, weighed with bone	1.0 medium por (150.0g)
13-525	Pepper, capsicum, red, boiled in unsalted water	1.0 each (160.0g)

Day 3

Breakfast

12315	Whole milk, average	280.0 g
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Lunch

A20113	Noodles, chinese, chow mein	2.0 1.5oz (88.0g)
A07059	Polish sausage, pork	1.0 sausage (227.0g)

Dinner

18-288	Pork, mince, stewed	1.0 medium por (100.0g)
13-173	Broccoli, green, frozen, boiled in unsalted water	1.0 medium por (85.0g)
11-880	Rice, white, basmati, easy cook, boiled in unsalted water	285.0 g

Grams Total 3773.0 g

Nutrient totals

Divisor = 3.00

COMA 1991 EAR, Males 19-50 years, PAL=1.4

Nutrient	Amount	EAR	%EAR
Water	920.40g		
Alcohol	0.00g	#	
Total Nitrogen	10.31g	#	
Protein	77.01g		44.40 173.45
Fat	65.04g		58.69 110.44
Carbohydrate	186.25g		201.29 92.52
Energy kcal	1808.07		2550.00 62.98
Energy kJ	6759.32		10600.00 63.77
Total Sugars	25.85g	#	
Glucose	2.59g	#	
Fructose	3.38g	#	
Sucrose	4.85g	#	
Maltose	0.47g	#	
Lactose	12.65g	#	
Galactose	0.00g	#	
Total Saturates	24.21g		17.85 135.65
Total Monounsaturate	27.25g		21.41 127.24
Total Polyunsaturate	8.70g		10.71 81.23
Cholesterol	227.25mg	#	
Tot Trans Fatty Acid	0.88g	#	3.57 24.03
Tot br-chain FA	0.02g	#	
Total n3 fatty acid	0.27g	#	0.36 74.53
Total n6 fatty acid	2.24g	#	1.78 125.29
AOAC fibre	2.98g	#	
Fibre (Soufngate)	0.00g	#	
Fibre (Soufngate)	7.03g	#	
Non-starch Polysacch	7.03g	#	
Cellulose	0.00g	#	
Starch	138.83g	#	
Resistant Starch	0.00g	#	
Lignin	0.00g	#	
Insoluble Polysacch.	0.00g	#	
Soluble Polysacch.	0.00g	#	

Energy Breakdown

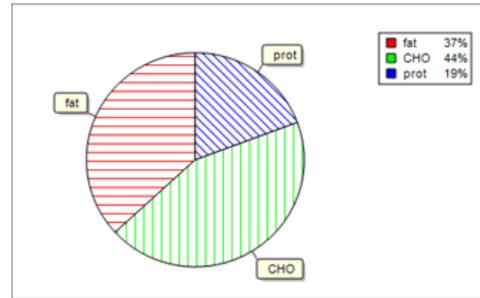
Divisor = 3.00

Nutrient	Quantity	Energy(kcal)	Percentage	ConvFact
Protein	77.01	308.06	19.18	4.00
Carbohydrate	188.25	698.42	43.49	3.75
Fat	65.04	585.33	36.44	9.00
Alcohol		0.00	0.00	7.00
Calculated Total:		1591.81	99.11	
Composition of CHO:				
Sugars	25.88	98.98	6.04	3.75
Starch	138.83	520.61	32.42	3.75
Composition of Fat:				
SFA	24.21	217.87	13.57	9.00
MUFA	27.25	245.22	15.27	9.00
PUFA	8.70	78.28	4.87	9.00

Ratios:

$\frac{p:s}{Na:K} = 0.36$
 $\frac{Na:K}{Na:K} = 0.82$

% energy from Fat, CHO, Protein & Alcohol



Day 2

Code	TotBc g	Totn3 g	Totn6 g	AOAC g	SFibre g	NSP g	Cellu g	Starch
Breakfast								
12 12315	N	N	N	N	N	0.00	N	0.00
Mid-morning snack								
13 17-169	N	N	N	N	N	0.00	N	0.00
14 11-807	0.00	0.01	0.34	0.32	N	0.47	N	5.63
Lunch								
15 11-450	0.00	0.07	1.19	N	N	6.65	N	107.45
16 18-288	0.02	0.17	1.39	N	N	0.00	N	0.00
17 15-871	N	N	N	N	N	0.90	N	0.00
Dinner								
18 11-860	N	0.02	0.27	T	N	T	N	57.78
19 18-318	N	N	N	N	N	0.00	N	0.00
20 13-525	N	N	N	3.84	N	1.28	N	T
Day total	0.02	0.27	3.19	4.16	0.00	9.29	0.00	170.88

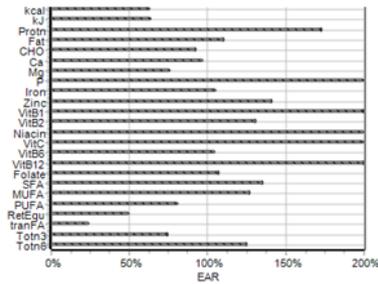
Day 3

Code	TotBc g	Totn3 g	Totn6 g	AOAC g	SFibre g	NSP g	Cellu g	Starch
Breakfast								
21 12315	N	N	N	N	N	0.00	N	0.00
Lunch								
22 A20113	N	N	N	N	N	3.18	N	N
23 A07059	N	N	N	N	N	0.00	N	N
Dinner								
24 18-288	0.02	0.17	1.39	N	N	0.00	N	0.00
25 13-173	N	N	N	N	N	3.06	N	0.68
26 11-880	N	0.03	0.43	T	N	T	N	91.49
Day total	0.02	0.20	1.82	0.00	0.00	6.24	0.00	92.17

Code	TotBc g	Totn3 g	Totn6 g	AOAC g	SFibre g	NSP g	Cellu g	Starch
List Total	0.06	0.80	6.71	8.88	0.00	21.08	0.00	416.49

N: nutrient is present but the value is not known
 T: trace
 E: nutrient is estimated

Graph of nutrient values as % of RV's
 COMA 1991 EAR, Males 19-50 years, PAL=1.4



Amino Acid Totals

Divisor = 3.00

Isoleucine	482.32	mg	#
Leucine	814.17	mg	#
Lysine	839.90	mg	#
Methionine	288.78	mg	#
Cystine	118.80	mg	#
Phenylalanine	407.84	mg	#
Tyrosine	335.98	mg	#
Threonine	447.19	mg	#
Tryptophan	104.42	mg	#

Valine	513.70	mg	#
Arginine	689.92	mg	#
Histidine	335.98	mg	#
Alanine	682.51	mg	#
Aspartic Acid	978.37	mg	#
Glutamic Acid	1601.88	mg	#
Glycine	750.61	mg	#
Proline	538.75	mg	#
Serine	441.89	mg	#

* : Approximate nutrient values used for some food(s)
 # : Unknown values for some food(s) treated as zero

Fatty Acid Totals

Divisor = 3.00

Fatty Acid	Systematic Name	Total	Status
1 4:0	#	butanoic	0.01
2 6:0	#	hexanoic	0.00
3 8:0	#	octanoic	0.00
4 10:0	#	decanoic	0.07
5 12:0	#	dodecanoic	0.07
6 14:0	#	tetradecanoic	0.48
7 15:0	#	pentadecanoic	0.00
8 16:0	#	hexadecanoic	8.87
9 17:0	#	heptadecanoic	0.11
10 18:0	#	octadecanoic	4.33
11 20:0	#	eicosanoic	0.02
12 22:0	#	docosanoic	0.01
13 24:0	#	tetracosanoic	0.00
14 10:1	#	decanoic	0.00
15 12:1	#	dodecenoic	0.00
16 14:1	#	tetradecenoic	0.01
17 15:1	#	pentadecenoic	0.00
18 16:1	#	hexadecenoic	1.02
19 17:1	#	heptadecenoic	0.03
20 18:1	#	octadecenoic	16.99
21 18:1cn7	#	cn7 octadecenoic	0.38
22 18:1cn9	#	cn9 octadecenoic	4.60
23 20:1	#	eicosenoic	0.10
24 22:1	#	docosenoic	0.01

Microdiet Version 4 for Windows - [E:\Clinical Trials\Micro-diet info\RD_3.fl2]

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Selected foods

Code	Foodname	Grp	Quant	Grams	Meal	Day
1 11-742	Breakfast cereal, cornflakes, fortified	AI	35 g	35	B	1
2 12312	Semi-skimmed milk, average	BAH	0.5 med glass	100	B	1
3 17-169	Tea, infusion, average, with semi-skimmed milk	PAC	0.2 oz	5.67	M	1
4 11468	White bread, sliced	AF	1 LgMedSlice	36	L	1
5 12346	Cheddar cheese	BL	1 gratedTbsp	10	L	1
6 5-858	Apples, eating, average, raw, peeled	FA	1 small	67	L	1
7 13453	Lettuce, average, raw	DG	28 mg	0.03	L	1
8 12312	Semi-skimmed milk, average	BAH	2 medium po	60	L	1
9 13434	Lentils, red, split, dried, boiled in unsalted water	DB	10 g	10	D	1
10 11468	White bread, sliced	AF	1 LgThkSlice	44	D	1
11 19080	Pork sausages, chilled, grilled	MI	0.5 thin	10	D	1
12 17486	Butter, spreadable	OA	2 teaspoon	10	D	1
13 5-744	Broccoli, green, raw	DG	1 spear	45	D	1
14 12312	Semi-skimmed milk, average	BAH	0.5 med glass	100	D	1
15 11-746	Breakfast cereal, cornflakes, crunchy / honey nut coated,	AI	35 g	35	B	2
16 14-329	Orange juice, chilled	FC	1 medium po	150	B	2

Foodlist total % energy

FoodList UserSet 1-12 13-24 25-36 37-48 49-60 61-72 73-84 Amin(e) Amin(i) SFA MUFA PUFA Phytos

Enter food code or food name string Search Day Code Meal Code Quantity

Add to List Remove Exchange Combine

All foods in Dataset

Code	FoodName	Grp
11-001	Arrowroot	AA
11-002	Barley, pearl, raw	AA
11-003	Barley, pearl, boiled	AA
11-006	Buckwheat, groats	AA
11-011	Commeal, sifted	AA
11-016	Flour, millet, foxtail	AA
11-021	Flour, rice	AA
11-023	Sago, raw	AA
11-027	Tapioca, raw	AA
11-069	Breadcrumbs, retail	AF
11-076	Bread, currant	AF
11-077	Bread, currant, toasted	AF
11-091	Bread, rye	AF

Portion size or weight

g (1.0)
oz (28.35 g)
kg (1000 g)
mg (0.001 g)
lb (453.6 g)
CHOX (10.64 g)
medium por (30.00 g)
heapedTbsp (30.00 g)
level Tbsp (20.00 g)

Nutr per 100g food

Water g 12.20
N g 0.07
Prot n g 0.40
Fat g 0.10
CHO g 94.00
kcal 355.00
Sugars g 0.00
SFA g 0.00
MUFA g 0.00
PUFA g 0.00
NSP g 0.10
Na mg 5.00

food %energy food description

Select dataset: UK 2015 CoFIDS (7th Edn) Print Preview Export to Text Help Close

Nutrient Totals v RV Nutrients by meal/day Nutrients in 100g Energy and Ratios Amino Totals

Nutnam	Units	NutTot	DivTot	Status
1 Water	g	7947.31	2649.1	
2 Alcohol	g	0	0	#
3 Total Nitrogen	g	32.88	10.96	#
4 Protein	g	203.67	67.89	
5 Fat	g	179	59.67	
6 Carbohydrate	g	1056.54	352.18	
7 Energy kcal		6408.07	2136.02	
8 Energy kj		27061.41	9020.47	
9 Total Sugars	g	444.7	148.23	#
10 Glucose	g	34.54	11.51	#
11 Fructose	g	36.45	12.15	#
12 Sucrose	g	302.19	100.73	#
13 Maltose	g	13.54	4.51	#
14 Lactose	g	44.44	14.81	#
15 Galactose	g	0	0	#
16 Total Saturates	g	80	26.67	#
17 Total Monounsaturat	g	48.46	16.15	#
18 Total Polyunsaturate	g	32.66	10.89	#
19 Cholesterol	mg	455.9	151.97	#
20 Tot Trans Fatty Acid	g	3.48	1.16	#
21 Tot br-chain FA	g	0.03	0.01	#
22 Total n3 fatty acid	g	0.89	0.3	*#
23 Total n6 fatty acid	g	15.3	5.1	*#
24 AOAC fibre	g	26.55	8.85	#
25 Fibre (Southgate)	g	16.38	5.46	*#
26 Non-starch Polysacc	g	31.9	10.63	*
27 Cellulose	g	0	0	#
28 Starch	g	554.35	184.78	#
29 Resistant Starch	g	0	0	#